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In This Issue
Reviews & Comments

SF1 MUTATION IN HUMANS

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INTRODUCTION

The purpose of this lead article is to bring readers up to date on the phenotypes, genotypes, and pathogenesis of the steroidogenic factor (SF)1 mutation that pediatric endocrinologists encounter in their practices and to provide new insights into SF1 function in humans. Steroidogenic factor 1 (Sf1 in mice or SF1 in humans), also called Ad4BP or NR5A1, is a nuclear transcriptional factor that binds to target gene promoters as a monomer and recognizes a canonical half-site motif. Structurally, both Sf1 and SF1 have characteristic domains of nuclear transcriptional factors. These consist of a zinc finger DNA-binding domain, a ligand-binding domain, and an activation function-2 domain. There is also an accessory DNA-binding domain that confers binding site stability and specificity.

Originally, SF1 was isolated as a global regulator for P450 steroid hydroxylases.[1,2] SF1 was thought to be responsible for tissue-specific expression of these enzymes in the adrenals and gonads. Subsequent studies in vitro have shown that Sf1 and SF1 regulate a lot of genes involved in adrenal and gonadal development, sex differentiation, steroidogenesis, reproduction, and many other metabolic functions.[2,3] Thus, Sf1 and SF1 play pivotal roles in the development and function of multiple endocrine organs.

From The Editor's Desk

This issue of *GGH* Volume 24, Number 1 is only available on-line and will be not be printed and mailed due to budgetary constraints. However this issue is available either as a PDF file or a web page so you can file it and/or print it and keep it for your enjoyment and as a reference resource.

The current issue includes an excellent and timely review of the "SF1 Mutations in Humans" by Dr. Tomonobu Hasegawa, plus 19 reviews of current papers in the literature with comments by the editorial board. There are four reviews pertaining to growth hormone treatment including the consensus guidelines of adult growth hormone deficiency, two addressing growth of celiac patients, three pertaining to height related issues on quality of life, the in vitro fertilization children or the genetics of stature. There are also two reviews regarding the aortic dilatation and the uterine development of Turner patients. In addition the late effects of cancer survivors, hypopituitarism following traumatic brain injury, and diabetes and stroke in hypopituitarism are also reviewed. I also want to bring to your attention the reviews on the FTO gene in obesity and the monoallelic expression of autosomal genes. Finally there are two reviews of papers dealing with two frequent alterations in pediatric endocrine practices, namely metabolic syndrome in brothers of PCOS women and the ventricular function of congenital hypothyroidism in neonates.

The economic situation in the country is being reflected in our journal. The reduced funding for continuous medical education will only allow us to publish two electronic issues in 2008, unless there is a renewed commitment for sponsorships that will allow us to provide our readers with a high quality journal more frequently. We will continue to search for means and will appreciate your tax deductible contributions. You may do so on line (www.GGHjournal.com or PedsAcademics.org) and click *make a donation*, or you may send a check to Pediatric Sunshine Academics, 1040 Alston Rd., Santa Barbara, CA 93108.

Thank you for your support,
Fima Lifshitz, MD
Editor-in-Chief

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The murine Sf1 also orchestrates the development and function of multiple endocrine organs in vivo, judging from the striking, but complex phenotypes of its knockout mice. The Sf1 knockout mice showed adrenal and gonadal agenesis, impaired function of pituitary gonadotropes, and structural abnormalities of ventromedial hypothalamic nucleus (VMH).[2,4] All knockout mice died within 2 weeks due to adrenal insufficiency. Moreover, recently established tissue- or cell-specific Sf1 knockout mice clearly demonstrated in vivo the direct and pivotal function of Sf1 in Leydig cells, granulose cells, pituitary gonadotropes, and VMH.[5-7]

PHENOTYPES, GENOTYPES, AND PATHOGENESIS OF SF1 MUTATION IN HUMANS

The critical role of murine Sf1 in vivo strongly suggests the importance of SF1 in humans, prompting endocrinologists to identify patients with SF1 mutations. Initially, the rare 46,XY patients that showed severe gonadal dysgenesis together with primary adrenal failure were the main focus to identify SF1 mutations. These alterations were analogous to the phenotypes of the knockout mice. Indeed, the first described human patient with SF1 mutation (a heterozygous G35E) was a 46,XY female who presented with primary adrenal failure in the first 2 weeks of life; she had a vascular collapse at 17 days of age. The phenotype of this patient was similar to those seen in Sf1 knockout mice, albeit less severe. This patient's serum cortisol was 1.2 g/dL and aldosterone was 5.0 ng/dL, both of which were quite low considering the clinical condition, together with a high plasma ACTH (1,165 pg/mL). She had been treated with glucocorticoids and mineralocorticoids. Before the induction of puberty as female, her pituitary gonadotropins responded to GnRH stimulation test: LH (1.2 → 8.6 mIU/mL) and FSH (17.8 →38.0 mIU/mL). No response of testosterone was observed by hCG stimulation test. At laparotomy, normal Mullerian structures and streak-like gonads were found. Histological examination of the gonads showed poorly differentiated tubules and connective tissue. Mutation analysis of SF1 revealed a heterozygous mutation in the proximal box (P-box) of the first zinc finger of SF1. The P-box is important for the recognition of DNA binding and confers specificity to nuclear receptors in the regulation of target genes. The mutant SF1 protein did not bind to a canonical SF1 binding site, did not transactivate the SF1 responsible gene, and did not exhibit dominant-negative effects.[8]

The next reported 46,XY patient with SF1 mutation (homozygous R92Q) was a normal female baby who presented one day after birth with a hypoglycemic convulsion due to primary adrenal failure.[9] Thereafter, the phenotypic spectrum of the SF1 mutation in humans has been strikingly expanded.[10-18] The phenotypes, genotypes, and pathogenesis of SF1 mutation in humans reported to date are summarized in Tables 1-3. A number of "milder form" 46,XY patients have also been reported. These patients had 46,XY disorders of sex development (DSD), namely, testicular dysgenesis (or impaired androgen production) with normal adrenal function. Six 46,XX subjects have been reported, all of whom had seemingly normal ovarian development and function; one out of the 6 had primary adrenal failure.

Table 1. Reported Cases of SF1 Mutation in Humans

Case	1	2	3	4	5	6
Age (years)	20	newborn	31	6	27	newborn
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY
Legal sex	Female	Female	Female	Female	Female	Female
Mutation	G35E/wild	R92/R92	1058-1065del/wild	C16x/wild	18delC/wild	V15M/wild
Clinical Features						
External genitalia	Normal female	Normal female	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Clitoromegaly	Normal female
Gonadal histology	Testicular dysgenesis	Not described	Testicular regression	Testicular dysgenesis	Testicular dysgenesis	Testis
Adrenal failure	Yes	Yes	No	No	No	No
Obesity	Yes	NA	Yes	Yes	Yes	NA
Sf-1 function of mutant allele (%)	0	0-50	0	0	0	0
Dominant negative effect of mutant allele	No	Not described	Yes	No	No	No
Total SF-1 function *in vivo* (%)	50	<50	0-50	50	50	50
Reference	8	9	10	11	12	13

These phenotypes in 46,XX subjects suggested sexual dimorphism in SF1 function in gonads.

THE IMPORTANT ROLE OF SF1 GENE DOSAGE

Heterozygous mutation of SF1 causing human disease has established the concept of a dose-dependent action of SF1 in vivo. In contrast, heterozygous Sf1 knockout mice show no variations in phenotype, although latent adrenal insufficiency has been unmasked under stressful conditions.[19] Nineteen identified patients are listed in Tables 1-3. All reported patients except case 2 had a heterozygous mutation of the SF1 gene. In cases 1 and also in cases 4 to 11, the mutant SF1 had null function without dominant negative effects. Thus, all of these 9 patients had 50% of total SF1 function in vivo. In cases 12 to 15, the mutant SF1 had null to 20% function. These 4 patients had 50% to 60% of total SF1 function. In case 18, the mutant SF1 only had a 55% function without dominant negative effects, suggesting that this patient had 77.5% of total SF1 function. It was of note that case 2 had a homozygous mutation of SF1. This patient had less than 50% of total SF-1 function in vivo, judging from the mutant SF-1 with 0% to 50% of total SF1 function. On the other hand, her parents and a brother had heterozygous mutations, thus these 3 members of the patient's family were phenotypically normal and had more than 50% of total SF1 function. Case 3 had heterozygous mutation. This mutation had null function together with dominant-negative effect. Therefore, case 3 had less than 50% of total SF1 function in vivo. Taken together, these reported patients have established the importance of dosage-dependent action of SF1 in humans.

"MILDER FORM" OF 46,XY PATIENTS

Sixteen out of 18 of the 46,XY patients reported were reared as female from birth (case 18 was suspected to have 46,XY although the karyotype was not described). Androgen production in fetal testis must therefore be insufficient. Four patients showed testicular dysgenesis or regression on macroscopic or microscopic examination. Conversely, only 2 patients (cases 1 and 2) showed adrenal failure. This suggests that in humans the testis might be more sensitive to a partial loss of SF1 function than the adrenal gland.

We described a 27-year-old Japanese woman with testicular dysgenesis without adrenal failure.[12] This woman never had an adrenal crisis, even at the time of infection. She had clitoromegaly, advanced virilization during pubertal age (such as voice breakage and hirsutism), and primary amenorrhea. Her karyotype was 46,XY. Small masses were palpable bilaterally in the inguinal regions. Skin pigmentation was not observed and her plasma ACTH (21 pg/mL) and serum cortisol (13.4 g/dL) were normal. An ACTH stimulation test showed a normal response of cortisol (25.3 g/dL). Urine steroid profile by a gas liquid chromatograph/mass spectrometry indicated normal steroidogenic enzyme activities. Bilateral gonadectomy was performed, and histological examination of the gonads showed dysgenetic testes,

Table 2. Reported Cases of SF1 Mutation in Humans

Case	7	8	9	10	11	12
Age (years)	newborn	newborn	newborn	2	2	22
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY
Legal sex	Female	Female	Male	Female	Female	Female
Mutation	M78I/wild	G91S/wild	L437Q/wild	C55/wild	Delta395E/wild	R84C/wild
Clinical Features						
External genitalia	Normal female	Clitoromegaly urogenital sinus	Small phallus hypospadia chordee	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Slight clitoromegaly posterior labial fusion
Gonadal histology	Testis	Testis	Testis	Testis	Testis	Testis
Adrenal failure	No	No	No	No	No	No
Obesity	NA	NA	NA	NA	NA	NA
Sf-1 function of mutant allele (%)	0	0	0	0	0	10
Dominant negative effect of mutant allele	No	No	No	No	No	NA
Total SF-1 function in vivo (%)	50	50	50	50	50	55
Reference	13	13	13	14	14	15

Remarks
Case 7 Mother has M781/wild
Case 8 Mother has G91S/wild

severely hyalinized seminiferous tubules containing a few Sertoli cells, and loose interstitium containing a few Leydig cells. Molecular analysis of SF1 revealed a heterozygous single base pair deletion (18delC), theoretically leading to frameshift and early termination. Indeed, mutant SF1 failed to activate the target gene in transactivation analysis and did not have a dominant-negative effect.

The presence of "milder form" of 46,XY patients were again in contrast with XY heterozygous mice, the Sf1 knockout allele showed normal external genitalia, normal fertility, but latent adrenal insufficiency under stressful conditions.[19] Thus, species differences between mice and humans exist in terms of phenotypes due to loss of function of Sf1 or SF1.

SEEMINGLY NORMAL OVARIAN DEVELOPMENT AND FUNCTION IN 46,XX PATIENTS

In humans, there have been 6 cases of 46,XX reported with SF1 mutation. All 6 had seemingly normal ovarian development and function. Only one had primary adrenal failure. Case 20 was the first reported 46,XX patient with SF1 mutation.[18] This phenotypically normal 14-month-old girl developed adrenal insufficiency and seizures after otitis and tonsillitis. At that time, hyponatremia (serum Na 104 mmol/L), hyperkalemia (serum K 8.0 mmol/L), elevated plasma ACTH (2,200 pg/mL), and inappropriately low cortisol (165 nmol/L) indicated primary adrenal insufficiency. Serum LH and FSH were 0.5 mIU/mL and 2.8 mIU/mL at the age of 14 and 27 months, respectively. Imaging studies using pelvic ultrasonography and MRI confirmed the presence of bilateral ovaries of normal size. Thus, no evidence of abnormality of ovarian development and function were found.

Recently, the mothers of cases 7, 8, 12, 16, and 18 were reported to have the same mutation that was detected in the patients, indicating the ovarian development and function of these mothers were completely normal. Moreover, none of the mothers showed adrenal insufficiency. These 5 families suggested a sex-limited autosomal dominant inheritance of the SF1 mutation.

OBESITY IN ADULT PATIENTS

Four out of 5 of the 46,XY adult patients with an SF1 mutation had obesity. Thus, obesity might be part of the phenotype of SF1 mutation in humans. A partial loss of SF1 function in the VMH in humans may lead to obesity. The presence of obesity was consistent with mice studies. Majdic et al[20] rescued Sf1 knockout mice with corticosteroid injections, followed by adrenal gland transplantation. These transplanted mice had indistinguishable ACTH and corticosterone levels to wild-type mice, indicating restoration of hypothalamic-pituitary-adrenal axis. With gonadoctomy, at earlier ages

Table 3. Reported Cases of SF1 Mutation in Humans

Case	13	14	15	16	17	18	19
Age (years)	4	14	10	8	22	NA	1
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	NA	46,XX
Legal sex	Female	Female	Female	Female	Female	Male	Female
Mutation	C33S/wild	R84H/wild	Y138X/ wild	c1277dupT/wild	C424_427dupCCCA/ wild	V333M/ wild	R255L/ wild
Clinical Features							
External genitalia	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Normal female	Micro-penis anorchia	Normal female
Gonadal histology	Testis	Testis	Testis	Testis	Streak	Fibrous tissue	Ovary
Adrenal failure	No	No	No	No	No	No	Yes
Obesity	NA	NA	NA	NA	NA	NA	NA
Sf-1 function of mutant allele (%)	0-20	0-20	0-20	NA	NA	55	0
Dominant negative effect of mutant allele	No	No	No	NA	NA	No	No
Total SF-1 function in vivo (%)	50-60	50-60	50-60	NA	NA	77.5	50
Reference	16	16	16	16	16	17	18

Remarks
Case 16 Mother has c1277dupT/wild
Case 18 Mother has V355M/wild Phenotypically normal dizygotic twin brother has V355M/wild

the weights of transplanted mice did not differ significantly from the wild-type mice. Later in life, adrenal-transplanted Sf1 knockout mice developed obesity due to decreased spontaneous locomotor activity, rather than increased appetite. It was of note that obesity was considerably more severe in females, although the reason for this sexual difference was unknown. Sf1 and the VMH nucleus in the hypothalamus were thought to play important roles in metabolism rather than in appetite regulation.

Increased weight also occurred in CNS-specific Sf1 knockout mice fed a high-fat diet.[7] Considering the Sf1 expression in CNS, the responsible region of obesity must be VMH. CNS-specific Sf1 knockout mice showed decreased wheel running capacity before becoming obese, indicating that obesity was due to decreased spontaneous locomotor activity.

Brain-derived neurotrophic factor (Bdnf), which stimulates growth of neurons via TrkB receptor, is expressed in the hypothalamus including the VMH. CNS-specific Bdnf knockout mice also became obese. This raised the question of whether Bdnf was a direct target gene of Sf1 in VMH. However, CNS-specific Sf1 knockout mice developed obesity only when ingesting a high-fat diet, while adrenal-transplanted Sf1 knockout mice showed obesity when fed a regular diet. Some plausible explanations are possible for these differences. Subtle abnormalities in function of adrenal transplants in original Sf1 knockout mice may result in glucocorticoid excess. The presence of gonads in CNS-specific Sf1 knockout mice may ameliorate the effects of sex steroid deficiency. It should also be kept in mind that Cre-mediated disruption of Sf1 in CNS-specific Sf1 knockout mice at ~E14 may permit certain developmental events to occur before inactivation, in contrast to original Sf1 knockout mice. Most patients with SF1 mutation were children at the time of the study, thus long-term follow-up is necessary to ascertain if they develop obesity as adults.

UNSOLVED ISSUES OF SF1 MUTATION IN HUMANS

Some important issues regarding SF1 mutation in humans remain unsolved. First, it is not known if any "milder form" of 46,XY patients with DSD, and seemingly normal adrenal function, would eventually develop late-onset adrenal insufficiency. Thus, longitudinal follow-up of these patients is mandatory. Second, if "milder form" 46,XY patients with DSD persist with normal adrenal function even on long-term follow-up, why does the SF1 mutation cause testicular dysgenesis or impaired androgen production, but not adrenal insufficiency? Third, the full phenotypic spectrum of 46,XY SF1 mutation has not been documented. Previous publications have shown that most of the patients had 46,XY DSD without adrenal insufficiency. Additionally, one patient with SF1 mutation was described to have bilateral anorchia and micropenis.[17] He had a mild partial loss of SF1 function. Thus, 46,XY SF1 mutation may result in a wide spectrum of male reproductive phenotypes. Fourth, the molecular mechanism of sexual dimorphism of the SF1 phenotypes regarding gonads is not clear. The 46,XY patients showed testicular dysgenesis or impaired androgen production, whereas 46,XX patients showed an apparent normal ovarian development and function. SF1 might have different target gene(s) depending on developing fetal testis or ovary. Fifth, the molecular mechanism of the development of obesity in SF1 needs further clarification, although mice studies suggested the loss of VMH function.

SUMMARY

The identified patients with SF1 mutation definitively showed a critical role of SF1 function in vivo. There is a different functional importance of Sf1 (SF1) between mice and humans. In summary, in humans: (1) the important role of SF1 gene dosage has been elucidated; (2) a number of "milder form" of 46,XY patients have been reported with DSD and normal adrenal function; (3) 46,XX patients had seemingly normal ovarian development and function; and (4) adult patients might develop obesity.

Acknowledgements

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Letter to the Editor
Caucasian or White Phenotype?

The term Caucasian is frequently employed to describe a white individual. Caucasian is used as a synonym for a group of people that share the common character of whiteness. It is frequently used by distinguished researchers when they analyze the differences between various ethnic groups in relation to a phenomenon they have studied. According to the definitions given by *The American Heritage Dictionary of the English Language*, "Caucasus or Caucasia is a region between the Black and Caspian seas that includes Russia, Georgia, Azerbaijan, and Armenia." Caucasian relates to the Caucasus region or its peoples, languages or cultures. It also refers to a major human racial division traditionally distinguished by physical characteristics such as very light to brown skin pigmentation and straight to wavy or curly hair, and including peoples indigenous to Europe, Northern Africa, Western Asia, and India. Thus there are dark and curly haired Caucasians, as there could be very white, light-eyed Latinos or very light to dark skin in other groups. I believe that what scientists who use the term Caucasian are trying to say is that the term refers to a Caucasian, especially of Nordic type or, at least, as defined by the dictionary, "White: A member of a racial group of people having light skin coloration, especially one of European origin." If so, why abandon the term white?

The term Caucasian may intend to reduce a great number of phenotypes into a group that shares other characteristics as well. In a superb, well documented article the significance of the term phenotype is discussed.[1] The current definition of phenotype is: "the complete observable characteristics of an organism or group, including anatomic, physiological, biochemical, and behavioral traits, as determined by the interaction of both genetic makeup and environmental factors." One realizes that an external character cannot imply, by itself, a necessary similarity between two or more individuals or groups. As the authors state, "The interaction of genes and the environment has the potential to produce a myriad of phenotypes." For example, is it not true that among the Caucasian population in the world there are those who differ greatly in skin hues, ethnicity, and genetic factors? On the other hand a white skinned, light-eyed Latino (or Hispanic as the US Census classifies) may have the appearance, genetic background, behavioral traits, and environmental influences of a Caucasian. If it is melanin that determines the grouping, why not just use the term white?

Cesar Chavarria, MD
Mexico City, Mexico

Reference

1. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Diabetes Care. 2007;Supplement 2:156-60.

Editor's Response: *Dr. Chavarria makes a good case to cease using the term Caucasian in describing white patients. The AMA Manual of Style states, "Racial categories should not be used automatically. Authors should explain and justify racial designators. Caucasian is occasionally used to indicate white but is technically specific for people from Caucasus region and thus should be avoided." For several years GGH has used the term white. Unfortunately, the classification of Hispanic and Latino is far more complicated and controversial.*

Fima Lifshitz, MD

REVIEWS & COMMENTS FROM THE LITERATURE

Dosing of Growth Hormone Therapy According to IGF Levels

Cohen and colleagues conducted a 2-year, open-label, randomized, insulin-like growth factor (IGF)-I concentration-controlled trial, administering varying doses of growth hormone (GH) to test whether IGF-I levels achieved during GH therapy are determinants of the growth responses to GH treatment. The 172 subjects (77% male) were pre-pubertal children (mean age 7.53 years) with short stature (mean height SDS –2.64, mean IGF-I SDS –3.56). Subjects were randomized to receive GH treatment following one of 3 regimens: (1) conventional GH dosing based on the patient's weight (40 mcg/kg/d, n=34); (2) regularly adjusted GH doses to achieve an IGF-I SDS of –0.5 to +0.5 ($IGF_{(low)}$ group, n=70) or; (3) regularly adjusted GH doses to achieve an IGF-I SDS of +1.5 to +2.5 ($IGF_{(high)}$ group, n=68). Groups did not differ significantly on demographic or baseline variables such as height, IGF-I levels, peak GH, or bone age.

Baseline data collected included concomitant illness and medications, physical examination, funduscopy, height, weight, determination of IGF-I, pubertal staging,

checks for scoliosis and slipped capital femoral epiphysis (SCFE), blood sampling, and urinalysis. Study visits occurred at months 0, 1, 3, and every 3 months thereafter until 2 years. Adverse event reporting, height, weight, IGF-I, funduscopy, vital signs, and physical examinations for scoliosis and SCFE were conducted at all repeat visits. Laboratory evaluations performed at baseline were repeated annually, and bone age x-rays were obtained at baseline and year 2. Analysis of covariance was used to test for treatment effects, using baseline height-SDS (HT-SDS) as a covariate. Of the 172 enrolled participants, 147 completed the study. An intent-to-treat statistical analysis was performed including all randomized patients who received GH and at least one post-baseline height and IGF-I assessment.

Dosage and Growth. All 3 treatment groups demonstrated increased HT-SDS scores at the end of the study (median of 24 months), with the $IGF_{(high)}$ group showing the greatest increase (1.58 SDS) compared with the $IGF_{(low)}$ group (1.08 SDS) and the conventional dosing group (1.00 SDS). Annualized growth velocities for the $IGF_{(low)}$, $IGF_{(high)}$, and conventional groups were 9.71, 11.20, and 9.01 cm/year at 12 months, and 8.38, 10.03, and 8.16 cm/year at 24 months, respectively. Mean IGF-I SDS showed a rapid increase in all 3 groups during the first month after initiation of GH treatment; the target IGF-I values were generally reached within 6 to 9 months. The $IGF_{(high)}$ group had a target IGF-I SDS value of 2.0 (1.5–2.5) and the $IGF_{(low)}$ 0 (–0.5 to 0.5). IGF-I SDS values for the $IGF_{(high)}$ group were significantly higher than for the $IGF_{(low)}$ and the conventional groups from 6 months onward; no differences were found between mean IGF-I SDS for $IGF_{(low)}$ and conventional groups. Mean daily GH doses for the 3 treatment groups were 110 (median 98, range 20 to 346) mcg/kg/day for the $IGF_{(high)}$ group, 33 (median 28, range 9 to 114) mcg/kg/d for the $IGF_{(low)}$ group, and 41 (median 41, range 34 to 45) mcg/kg/day for the weight-based GH dosing comparison group. The $IGF_{(high)}$ group received a substantially larger mean GH dose than the other 2 groups, but no significant differences in the mean dose between the $IGF_{(low)}$ group and the comparison group were found. For all participants, the change in HT-SDS from baseline was positively correlated with both the IGF-I SDS change from baseline and with the cumulative GH dose. Multivariate analysis revealed that height outcome was significantly related to treatment group (accounting for 42% of the variance), inversely related to baseline peak GH level (39%), and inversely related to baseline IGF-I SDS (15%).

Safety. Over the 2-years, treatment-emergent adverse events were reported in 95.7% of participants in the $IGF_{(low)}$ group, 86.6% of patients in the $IGF_{(high)}$ group, and 82.4% in the conventional treatment group; most commonly, upper respiratory tract infection, headache, fever, coughing, and injection site hematomas. There was no occurrence of intracranial hypertension or malignancy. There was one case of SCFE in the $IGF_{(high)}$ group and 11 cases of worsening scoliosis (3 in the conventional, 4 in the $IGF_{(low)}$ group, and 4 in the $IGF_{(high)}$ group). Change in fasting serum insulin levels from baseline in the $IGF_{(high)}$ group was significantly greater than in the other groups, although mean serum insulin remained within the normal range for all groups. Bone age was delayed by approximately 2 years in all 3 groups at baseline, and after 2 years of treatment, bone age showed an increase of 2.45 to 2.82 years with no differences identified among the 3 groups.

The authors concluded that the $IGF_{(high)}$ group, titrated to the upper portion of the normal range, demonstrated significantly greater height gains than the $IGF_{(low)}$ and conventional groups. Expressed in height benefit, the $IGF_{(high)}$ group gained approximately 3 cm more in height than the comparison groups after 24 months of GH treatment. The study lacked sufficient power to detect the safety of IGF-based dosing in terms of rare side effects. No information regarding the long-term safety of such a regimen, especially in terms of cancer risk, was provided.

Cohen P, Rogol AD, Howard CP, et al. Insulin growth factor-based dosing of growth hormone therapy in children: A randomized, controlled study. J Clin Endocrinol Metab. 2007;92:2480-6.

Editor's Comment: *This study provides evidence for the feasibility of IGF-based GH dose titration; however, the increased height gains compared to the conventional treatment dosing were only significant for the $IGF_{(high)}$ group. The authors were circumspect by restricting interpretation of the findings to a demonstration of the feasibility of IGF-I GH dose titration and not as a recommendation for clinical practice. Important considerations to explore before implementing such a strategy in regular practice include: (1) GH doses administered to this group were as high as 346 mcg/kg/day (mean 110), compared to the mean conventional weight-based dose of 41 mcg/kg/day; this represents as high as a 9-fold increase compared to previously studied values; (2) given the lack of safety data beyond the length of 2-year study, movement toward increasing GH above the conventional dosing should be discouraged. An editorial by Baron accompanying this paper stressed that the principle of primum non nocere dictates that weight-based dosing remain the standard of care.*[1]

Although there is a dearth of information to inform us about the possible negative side effects that may be associated with prolonged treatment with high doses of GH, there is certainly a theoretical basis for concern. A growing body of epidemiological data suggests that high levels of circulating IGF-I constitute a risk factor for the development of breast, prostate, colon, and lung cancer.[2] *This study by Cohen and colleagues demonstrated a height gain of 3 cm for the $IGF_{(high)}$ group compared to the $IGF_{(low)}$ and conventional weight-based dosing groups. Even if the substantial excess cost of the additional GH administration of higher dosages is not considered, does the potential (not guarantee) for taller adult height justify potentially increasing a child's risk of developing cancer?*

Baron reminds the reader that "risk must be weighed against benefit" and states that "although short stature may be quite unpleasant for some individuals and carry social disadvantages, it generally does not cause death, serious physical dysfunction, or probably even serious psychological dysfunction."[1] This opinion is grounded in empirical evidence.[3]

Baron also encourages careful evaluation of the etiology of short stature before prescribing a costly and invasive procedure to which greater than 80% of children experienced some adverse side effects. Although Cohen et al used GH therapy in children with GH deficiency as well as in children with other categories of non-GH deficient short stature, the situation may be more complex and different among the various types of patients. As an example, it is well known that decreased IGF-I levels reflect nutritional status, not necessarily GH deficits,[4] yet no attempts were made to distinguish patients who may have had nutritional growth retardation, nor were the body weights of the patients defined. It has been shown that a subgroup of children with idiopathic short stature show decreased weight for height,[5] which is not typical of GH deficiency, suggesting their decreased growth and IGF-I may reflect insufficient nutrition. In such cases, lifestyle and dietary changes would be a more expedient, safer, and cost-effective treatment for the child.[6]

David E. Sandberg, PhD

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IGFs and Cytokines in Celiac Disease

The interesting study reported in this paper is the result of one of the few productive collaborations between pediatric endocrinologists and their gastroenterologist colleagues. This endocrine group from Parma, Italy has already published papers on the interaction of the cytokine and insulin-like growth factor (IGF) systems in Crohn's disease and cystic fibrosis. Growth failure is a well known feature of childhood celiac disease, however the precise mechanisms are not established and the possible influences of pro-inflammatory cytokines have not been well explored. The patients studied had "atypical" celiac disease, ie, they presented after the classical period of infancy. These patients were not extremely short at diagnosis but BMI SDS was decreased and both height and BMI increased significantly after treatment with a gluten-free diet.

Baseline values of IGF-I were reduced compared to controls ($P<0.05$) and interlukin (IL)-6 and tumor-necrosis factor (TNF)-α values were significantly elevated. IGF binding protein (IGFBP)-2 acts as an acute phase protein and, as reported in inflammatory bowel disease and childhood malignancy, values were elevated in affected subjects compared to controls. On treatment with a gluten-free diet, IGF-I and IGFBP-3 normalized and IL-6 and TNF-α decreased significantly. This study provides indirect evidence that cytokines may be involved in the abnormalities in the IGF system and when mucosal inflammation is suppressed, as occurs with treatment of celiac disease, and leads to the increases of IGFs and IGFBP-3 which facilitate normalization of linear growth.

Street ME, Volta C, Ziveri MA, et al. Changes and relationships of IGFS and IGFBPS and cytokines in coeliac disease at diagnosis and on gluten-free diet. Clin Endocrinol (Oxf). 2008;68:22-8.

Editor's Comment: *The celiac disease debate remains as to whether it is improvement in nutrition or suppression of inflammation which drives the recovery of growth. Both factors probably contribute, however as shown in Crohn's disease,[1] suppression of inflammation can independently result in increase of serum IGF-I, therefore the contribution of active inflammation may be subtle, but should not be discounted.*

Martin O. Savage, MD

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Aortic Dilatation and Dissection in Turner Syndrome

The cardiovascular phenotype in Turner syndrome (TS) is largely defined on clinical signs such as aortic valve abnormalities and aortic coarctation. Investigation in asymptomatic patients has revealed a far more complex phenotype. Combined echocardiography and MRI have shown that up to 75% of adult women with TS have significant cardiovascular abnormalities. In parallel there have been reports of a high rate of aortic dissection in TS and dilation of ascending aorta could be among predisposing factors. It is still unknown whether aortic dilatation precedes dissection in these patients and what specific diameter predicts impending deterioration.

The purpose of this study by Matura et al was to reliably identify girls and women at risk for such acute aortic events. This study included 166 adult volunteers with TS, aged more than 18 years, who

were not selected for cardiovascular disease and a group of healthy females. Ascending and descending aorta diameters were measured by MRI at the right pulmonary artery. Average diameters were identical in both groups; however results needed to take into account a mean 20 cm difference in height between both groups. When normalized to body surface area (aortic size index) the ascending aortic diameters were significantly greater in the TS group, and close to 32% of the TS women had values >95th percentile of 2.0 cm/m^2. Ascending/descending aorta diameters ratios were significantly greater in the TS group. During 3 years of follow-up aortic dissection occurred in 3 women with TS. Their ascending aortic diameters ranged from 3.7 to 4.8 cm and the aortic size indices were >2.5 cm/m^2. This rate is almost 100 fold higher than that of normal women who are usually affected at a much later age. Unfortunately there are no prospective data to know whether dilatation of the ascending aorta preceded dissection or elongation of the transverse aortic arch—a feature more recently described in TS.

The risk for aortic dissection is greatly increased in young women with TS. Because of their small stature, ascending aorta diameters of >5 cm may represent significant dilatation. The use of an aortic size index is therefore recommended. Individuals with a dilated ascending aorta defined as aortic size index >2.0 cm/m^2 require close cardiovascular surveillance, and values >2.5 cm/m^2 indicate a high risk for aortic dissection. The authors suggested that haploinsufficiency for a pseudoautosomal gene is responsible for the linked cardiovascular and lymphatic defects in TS. In addition, it is acknowledged that this study did not provide evidence-based recommendations for the follow-up of these patients with aortic dilatation. Further studies are also needed, like those in Marfan syndrome, to determine whether beta-blocker or rennin-angiotensin system blockade may prevent or retard aortic dilatation and if prophylactic surgery is appropriate.

Matura LA, Ho VB, Rosing DR, Bondy CA. Aortic dilatation and dissection in Turner syndrome. Circulation. 2007;116:1663-70.

Editor's Comment: *Recently published clinical guidelines[1] for care of girls and women with TS recommended that magnetic resonance angiography be used, in addition to echocardiography to evaluate the cardiovascular system. It was suggested that patients with defined defects be cautioned in regard to pregnancy. The present study of Matura et al provided an interesting addition of a new tool with appropriate reference data, which should help to evaluate the vital risk of aortic dissection in TS. However, prospective studies are needed which should include adolescent girls as well. The handling of the infertility issues is critical. The patients with spontaneous puberty and apparent ovarian activity should be evaluated for additional risk factors, such as systemic hypertension. The large group of infertile TS patients who have been told that assisted pregnancy can be considered in adulthood should keep in mind there is a risk of fatal aortic dissection during pregnancy. The aortic diameter should be monitored and be part of the follow-up and be taken into account in the reproductive life during adulthood.*

Raphaël Rappaport, MD

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The Late Effects of Childhood Cancer Survivors

Modern therapies and supportive care have increased the number of the childhood cancer survivors (CCS); as well, there has also been an increase in the late effects such as endocrine impairments and neuropsychological problems. These late effects often do not become clinically apparent until decades after cancer therapy. Unfortunately, over time the likelihood of medical follow-up decreases. Therefore, it is important for physicians to be aware of the late effects facing this population over their lifetime and the need to recall CCS patients for follow-up. However, where and by whom the follow-up of CCS can best be done is still a question that remains to be answered. Dickerman has set forth the recommendations for monitoring the late effects of CCS. He listed in a table both radiation-therapy site and chemotherapeutic agents along with the late effects that result from their use. These include: hypopituitarism, growth problems, hypogonadism, neurocognitive defects, coronary artery disease, caridiomyopathy, lung fibrosis, interstitial pneumonitis, breast cancer, nephropathy, muscle atrophy, osteoporosis, and second cancers. He recommended that in addition to being followed by a primary care physician, all CCS patients should also attend a specialized late-effects clinic on a yearly basis. At that specialized clinic, CCS patients would be evaluated by a member of the oncology team and subspecialists such as an endocrinologist, psychologist and neurologist. Ideally, such clinics should be located in the same center in which the patient was initially treated and be available on or near the site of residence.

Dickerman JD. The late effects of childhood cancer therapy. Pediatrics. 2007;119:554-68.

Editor's Comment: *This is a very special review article which provides important information for*

physicians who care for CCS patients. The survival rate of childhood cancer patients has markedly improved, thus the long-term late effects, such as endocrine impairments and neuropsychological problems, have become increasingly important. These alterations may result many years after conclusion of the cancer treatment. Currently, 10 million individuals in the US are living with a cancer diagnosis, 3 times the number of survivors in decades past. In the near future 1 of 450 individuals in the population will be a long-term CCS. The 5-year survival rate of children with cancer is 80% to 85%; presently 1 in 640 individuals between 20 and 39 years of age is a CCS. Approximately 270,000 in the US present long-term morbidity of CCS.

In another paper, Oeffinger et al[1] recently reported the chronic health conditions (late effects) in adults following the treatment of childhood cancer. Their retrospective cohort study tracked the health status of adults who received a diagnosis of childhood cancer between 1970 and 1986 and compared the results with those of siblings of the patients. They calculated the frequencies of chronic conditions in 10,397 survivors and 3034 siblings (mean ages 26.6 years and 29.2 years, respectively, at the time of the study). In 62.3% of the cancer survivors there was at least one chronic condition; 27.5% had a severe or life-threatening condition. The adjusted relative risk of a chronic condition in a survivor, as compared with siblings, was 3.3 (95% CI, 3.0 to 3.5); for a severe or life-threatening condition, the risk was 8.2 (95% CI, 6.9 to 9.7). Among survivors, the cumulative incidence of a chronic health condition reached 73.4% (95% CI, 69.0 to 77.9) 30 years after the cancer diagnosis, with a cumulative incidence of 42.4% (95% CI, 33.7 to 51.2) for severe, disabling, or life-threatening conditions or death due to a chronic condition (Table). Thus, CCS have a high rate of illness owing to chronic health conditions that occurred long after the cancer was treated. There are many long-term CCS who were treated in the last 50 years, and these patients still need monitoring.

The late effects resulting from current treatment will likely decrease with improved radiotherapy being delivered with newer equipment in better fractionation schedules, along with the replacement of, or the use of, reduced doses of second-cancer-inducing chemotherapy. However, new cancer therapies used now or in the future will, in all likelihood, be associated with their own late effects. The patients who are treated with these new therapies must also be monitored closely to assess the magnitude of any late effects. It is necessary for physicians, as well as patients and family members, to know that late effects of a cancer survivor can occur even after many years following cancer treatment. The signs and symptoms of late effects of CCS are often nonspecific and may be masked by the sequela of chemotherapy, radiation therapy, and/or surgery, and may not be clinically evident until much later in life. Therefore, they are likely to be overlooked if late effects are not actively searched for through regular follow-up. In previous issues of GGH there were 4 reviews of papers dealing with the long-term complications of CCS addressing height,[2] premature menopause,[3] growth hormone therapy and secondary neoplasms,[4] growth hormone deficiency, quality of life and neuropsychological function.[5] A clinic based model for survivors of childhood cancer has been proposed by Hinkle et al.[6]

Yoshikazu Nishi, MD

Relative risk of selected severe (grade 3) or life-threatening or disabling (grade 4) health conditions among cancer survivors, as compared with siblings.

Condition	Survivors (N=10,397)	Siblings (N=3034)	Relative Risk (95% CI)
	percent		
Major joint replacement*	1.61	0.03	54.0 (7.6–386.3)
Congressive heart failure	1.24	0.10	15.1 (4.8–47.9)
Second malignant neoplasm†	2.38	0.33	14.8 (7.2–30.4)
Cognitive dysfunction, severe	0.65	0.10	10.5 (2.6–43.0)
Coronary artery disease	1.11	0.20	10.4 (4.1–25.9)
Cerebrovascular accident	1.56	0.20	9.3 (4.1–21.2)
Renal failure or dialysis	0.52	0.07	8.9 (2.2–36.6)
Hearing loss not corrected by aid	1.96	0.36	6.3 (3.3–11.8)
Legally blind or loss of an eye	2.92	0.69	5.8 (3.5–9.5)
Ovarian failure‡	2.79	0.99	3.5 (2.7–5.2)

* For survivors, major joint replacement was not included if it was part of cancer therapy.
† For both groups, this category excludes basal-cell and squamous-cell carcinoma (grade 2). For siblings, this category includes a first cancer.
‡ Values are for women only.

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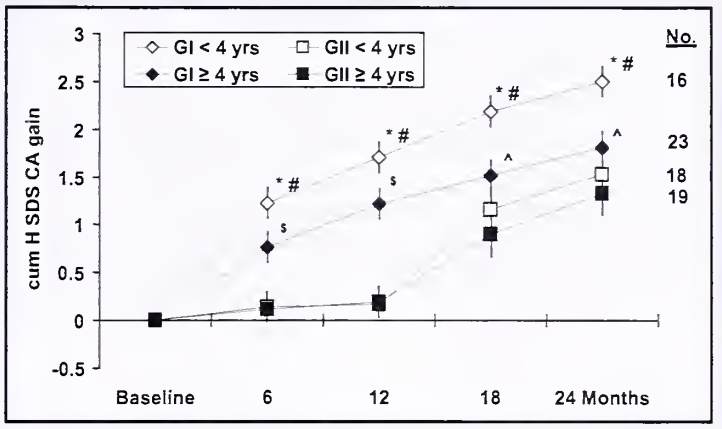
Growth Hormone Treatment in Very Young Children Born Small for Gestational Age

Argente and colleagues analyzed the outcome of growth hormone (GH) treatment in a large group of very young children born small for gestational age (SGA). They evaluated 76 children, recruited from 14 public hospitals in Spain, aged 2 to 5 years (37 males and 39 females; 45% less than 4 years of age) born SGA without catch-up growth during their first 2 years of life. The results after 24 months of GH treatment (0.06 mg/kg/day for 2 years, group I) were compared with those of a control group without treatment for 12 months, followed by 12 months of GH therapy (group II). The mean height SDS gain for chronological age in group I children was 2.10, compared to 1.43 in the children of group II. Height SDS for bone age was significantly different between groups only when group II did not receive GH treatment. Growth velocity SDS increased from –2.2 at baseline to 4.7 at 12 months in group I, while no significant changes from baseline values were noted in untreated group II subjects. Children in both groups under 4 years of age had the greatest gain in growth velocity, not only in SDS but also in their absolute increase in centimeters; weight SDS followed the same pattern (Figure). The BMI SDS did not change significantly during the study period and there was no significant acceleration of bone age. Fasting blood glucose, insulin, and HbA_{1c} levels remained within the normal range and with no difference among groups throughout the study. Both insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 increased significantly after 6 months of GH therapy and remained at a similar level thereafter, but did not exceed +2 SDS for chronological age during the study period.



Evolution of the SDS of height for chronological age (cumulative H SDS CA gain) in children less than or greater than 4 years of age in each group. p<0.05 group I (GI)<4 years vs GI >4years. ^p<0.05 GI >4 years vs. Group II (GH) >4 years. # p<0.05 GI <4 years vs. GH < and > 4 years. $ p<0.05 GI >4 years vs. GH <and>4 years. Group I received GH from the beginning of the study. Group II received GH starting at the 12 month time-point. (Mean and 95% confidence interval).
Reprinted with permission from Argente J, et al. J Clin Endocrinol Metab. 2007;92:3095-101. Copyright © Endocrine Society 2007. All rights reserved.

Argente J, Gracia R, Ibañez L, et al, on behalf of the Spanish SGA Working Group. Improvement in growth after two years of growth hormone therapy in very young children born small for gestational age and without spontaneous catch up growth: Results of a multicenter, controlled, randomized, open clinical trial. J Clin Endocrinol Metab. 2007;92:3095-101.

Editor's Comment: *Nearly 3% of infants are born SGA–that is with a weight and/or length at least 2 SD below the mean for gestational age. Most of these children undergo catch-up growth, allowing them to reach normal height by 2 years of age. However, close to 10% of SGA children fail to achieve an appropriate catch-up growth and remain short throughout childhood with a height below –2 SD. As demonstrated by a number of recent studies,[1,2] when treated with GH these patients can normalize their height during childhood, are able to maintain a normal growth velocity while prepubertal and during puberty, and can attain a normal adult height. Treatment with GH seems to be useful even in non-GH deficient SGA children and in those in whom no detectable cause for the lack of catch-up growth can be detected. However, most studies performed so far have been completed in older SGA children, so that the safety and efficacy of GH treatment in young children born SGA is unknown. In this study by Argente et al it was*

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demonstrated that very young SGA children with no spontaneous catch-up growth during the first 2 years of life are able to significantly increase their growth velocity and their height SDS following 2 years of GH therapy. However, continuously high plasma IGF-I and IGFBP-3 levels during therapy were evident. If these persist for years there may be potentially harmful effects.[3] The increase in growth velocity was greatest in SGA patients. Whether early GH treatment will result in a significantly greater adult height in these patients remains to be determined by long-term follow-up, but these observations seem to suggest the benefits and safety of early GH therapy in short children born SGA.

Roberto Lanes, MD

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Metabolic Syndrome in Brothers of PCOS Women

Polycystic ovary syndrome (PCOS), defined by hyperandrogenism, chronic anovulation, and/or polycystic ovary disease is one of the most common endocrinopathies in young women and evidence supports a central role of insulin resistance in the pathophysiology. Although previous studies have found high incidences of familial clustering of PCOS, suggesting that it might be a genetic disease, the lack of a male phenotype has made it difficult to assess the genetic component of this disorder. To date no study has looked at the gold standard of euglycemic-hyperinsulinemic clamp methodology in brothers of PCOS women. Baillargeon and Carpentier studied 17 brothers of women with PCOS and 28 male controls. The male controls selected were of comparable age and BMI as the brothers, and had no first-degree relatives with PCOS. Participants in the study were between 18 to 40 years of age with BMIs between 19-40 kg/m^2. The study protocol included anthropometric measurements of waist circumference and fasting blood samples for steroid levels. A standard OGTT was performed 2 days prior to a standard euglycemic-hyperinsulinemic clamp. In a subgroup of participants rates of oxygen consumption were measured during a 40-minute baseline period and during the last 40 minutes of the clamp to determine total body carbohydrate oxidation using indirect calorimetry. Assays obtained included total testosterone, androstenedione, DHEAS, 17 α-hydroxyprogesterone, sex-hormone binding globulin (SHBG), free testosterone, estrogen, progesterone, FSH, LH, thyrotropin, insulin, TSH, and prolactin. In addition C-reactive protein, total cholesterol, triacylglycerol, and HDL cholesterol were measured and LDL cholesterol was calculated. Fibrinogen, plasminogen activator inhibitor (PAI)-1 and factor VIII levels were also measured. For each variable the difference between the groups was adjusted for age and BMI using multiple linear regression analysis.

Age, BMI, waist circumference, and total fat percentage were comparable between the 2 groups as were systolic and diastolic blood pressure. Free testosterone levels, androstenedione, and DHEAS were similar in both groups. Fasting triacylglycerol levels were significantly increased in the brothers, but HDL and LDL cholesterol were comparable. PAI-1 and factor VIII were significantly increased in the brothers as was fasting glucose, 2-hour glucose levels and area under the glucose curve. Those with metabolic syndrome, as defined by the US National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III, were 18% in the brothers and 7% in the controls. This difference was not statistically significant. Insulin sensitivity was significantly decreased by 38% in the brothers and insulin stimulated total body carbohydrate oxidation was decreased by 65% in the brothers. After adjustment for age and BMI, the factor VIII levels, 2-hour glucose, as well as $AUC_{glucose}$ and $AUC_{insulin}$ during the OGTT were still significantly different between the 2 groups, but the differences in triacylglycerol, PAI-1, and fasting glucose were no longer significant. Those individuals with BMI >26.5 kg/m^2 had significantly increased PAI-1 and $AUC_{insulin}$ and significantly decreased androstenedione and sex hormone binding globulin.

The authors concluded that the brothers of women with PCOS have a significant decrease in insulin sensitivity associated with decreased glucose tolerance and hypercoagulability as evidenced by increased PAI-1 and factor VIII levels. Additionally, insulin stimulated glucose disposal was decreased by 65%. Thus brothers of women with PCOS displayed insulin resistance, glucose intolerance, and many of the characteristics of insulin resistance syndrome. A unique finding of the study was that glucose intolerance in the brothers of women with PCOS is irrespective of their degree of obesity. The authors noted that the limitations of their studies included a somewhat limited recruitment of all brothers since it was not possible to recruit all members of the affected and non-affected families. However, they concluded that their data suggest that brothers of PCOS women may have inherited insulin resistance and metabolic syndrome typical of PCOS and that these young men deserve careful clinical evaluation and long-term follow-up.

Baillargeon JP, Carpentier AC. Brothers of women with polycystic ovary syndrome are characterized by impaired glucose tolerance, reduced insulin sensitivity and related metabolic defects. Diabetologia. 2007;50:2424-32.

Editor's Comment: *Over the past several years pediatric endocrinologists have been evaluating an increasing number of adolescents with PCOS. It is not uncommon for these teenagers to be accompanied by mothers and sisters who also have obvious signs suggestive of PCOS. It is uncommon, at least in this editor's experience, for brothers of these teenagers to accompany them to the clinic visit. Thus, there is a potentially large group of teenagers and young adults with significant metabolic abnormalities who are not being evaluated and counseled. With the growing epidemic of obesity one is hesitant to suggest that pediatric endocrinologists actively recruit additional overweight adolescents to their clinics. However, the information presented above by Baillargeon and Carpentier suggests that to exclude the discussion of brothers' health status during the evaluation of girls with PCOS may be a significant omission. It would be of interest to obtain additional clinical information on brothers of adolescents with PCOS. This would appear to be an area for further clinical research.*

William A. Clarke, MD

Consensus Guidelines for Adult Growth Hormone Deficiency 2007

Ten years after the Growth Hormone (GH) Research Society drafted its "Consensus Guidelines for the Diagnosis and Treatment of Adults with Growth Hormone Deficiency (GHD)", a second international workshop was convened (Sydney, Australia) with 30 delegates to create an updated set of guidelines in 2007. Diagnosis of adult GHD was expanded in patient scope since the first document. Testing should be reserved for patients with evidence of hypothalamic-pituitary disease and with intention to treat. This includes patients with signs and symptoms of hypothalamic-pituitary disease (endocrine, structural, or genetic causes), patients who had received cranial irradiation or tumor treatment, and a new group, patients who had sustained traumatic brain injury or subarachnoid hemorrhage. Of note, the degree of pituitary dysfunction does not correlate with the severity of brain injury, and GH testing should be deferred for at least 12 months after injury due to the rate of endogenous GH axis recovery.

Another new patient group discussed is the GHD patient during the transition period, that newly recognized life stage between cessation of statural growth (ie, epiphyseal closure) and acquisition of complete somatic maturation (ie, full development of lean body mass and bone mineralization). Apart from patients with known genetic causes of GHD/hypopituitarism and patients with multiple pituitary hormone deficiencies (who should continue GH treatment without the need for further testing), patients with childhood onset GHD should undergo reevaluation of their GH function after at least one month off GH treatment for assessment of potential treatment during the transition period. A second reevaluation may be considered at the end of the transition period (about age 25) for those with isolated idiopathic GHD or discordant testing (low insulin-like growth factor [IGF]-I but normal stimulated GH peak) at the start of transition. Adult GH treatment is not indicated for patients with non-GHD pediatric indications, such as those born small for gestational age or Turner syndrome.

Adult GH treatment aims to "...correct the metabolic, functional, and psychological abnormalities associated with adult GHD." Dosing should be based on age and gender, not body weight, and escalated to response in a gradual and individualized fashion. Recommended monitoring of response includes:

1. Anthropometry (including weight, height, BMI and waist circumference): at least yearly
2. Quantified body composition and bone mineral density (DEXA): at baseline and every 2 years thereafter
3. Serum marker for GH dose titration (serum IGF-I): at least yearly and no sooner than 6 weeks after a dose change
4. Cardiovascular risk factors (blood pressure, fat mass, cholesterol panel): yearly, with similar goals as the general population
5. Fasting serum glucose: yearly
6. Quality of life (careful history, not disease-specific quality of life questionnaires)

Although GH treatment is indicated for adult patients with proven GHD, GH supplementation is not recommended for the physiologic age-related decline in GH/IGF secretion. Lower doses are called for in the elderly, to reduce the incidence of side effects and maintain age-dependent normal levels of IGF-I.

Ho KKY on behalf of the 2007 GH Deficiency Consensus Workshop Participants. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. Eur J Endocrinol. 2007;157:695-700.

First Editor's Comment: *The reader is encouraged to go through the entire original document, as the guidelines were too extensive to summarize here; this abstract highlights the newer recommendations. Also covered are the dosing recommendations, interactions with other hormone deficiencies, and treatment safety issues.*

More than the advances, what struck me were the

persistent unknowns of the field. Ten years after the first set of guidelines, we remain prey to suboptimal diagnostic testing. The lack of standardization of the GH and IGF-I assays was lamented in the consensus statement, as was the need for better age- and gender-related normative data. There was an entire section devoted to the various GH stimulation tests, their respective indications and limitations, and the multiple cut-off levels which also need better substantiating normative data. It is not surprising that the authors concluded, "...partial GHD is not adequately defined." Unless we can accurately distinguish normal from abnormal hormone levels, how can clinical care and research in the growth field advance effectively?

Adda Grimberg, MD

Second Editor's Comment: *The reader is encouraged to review the article in its entirety. However it may be worth noting a few more pertinent points in addition to those elaborated above. The consensus of experts stated that one stimulation test was sufficient for the diagnosis of adult GHD. They endorsed the use of an insulin or a glucagon tolerance test, and did not recommend clonidine, L-DOPA or arginine. GH releasing hormone (GHRH) + arginine or GHRH + GH-releasing peptide (GHRP) have also been validated, though GHD of hypothalamic origin may be missed, particularly in patients treated with cranial irradiation, then insulin or glucagon tolerance test may be necessary. The peak GH level for diagnosis was <3 mcg/L after insulin, higher levels may be acceptable following GHRH in individuals with a BMI of <25 kg/m^2. Measurements of circulating IGF-I levels constitute a good screen, though a normal level may not rule out GHD. Sex steroid, glucocorticosteroid and thyroid replacement should be optimized before testing or initiating GH treatment. The efficacy of treatment should be monitored and objective parameters determined, ie, body composition. Where available, DEXA should be utilized to quantitate body composition changes. IGF-I levels are indicted for titration of the GH dosages. Disease-specific quality of life questionnaires that assess the problems need to be validated.*

Fima Lifshitz, MD

Genetics of Stature

Variation in adult height is a classic polygenic trait, ie, it is determined by many genes each having a small effect. The identity of these genes has been elusive despite delineating many genes that have a major impact on height based on detection of mutations that cause severe growth deficiency. Although linkage studies have pointed to several genomic regions that influence height, there have not been any examples of gene variants that are reproducibly associated with height variation in the general population. However, from analysis of genome-wide association data, Weedon et al now showed that common variants in the *HMGA2* oncogene are associated with height.

The investigators began by analyzing data from 4,921 individuals including 1,896 UK individuals with type 2 diabetes from the Wellcome Trust Case Control Consortium and 3,025 Swedish or Finnish participants from the Diabetes Genetics Initiative. More specifically, they performed a meta-analysis of sex- and age-adjusted height z–scores for 364,301 autosomal single nucleotide polymorphisms (SNPs) common across data sets. These SNPs provide 64% coverage of the Utah-based Haplotype Map.

Two SNPs most associated with height were mapped in and 12 kb downstream of the 3' UTR (3' untranslated region) of the high mobility group-A2 (*HMGA2*) gene. *HMGA2* is a strong biological candidate for influencing height because its homozygous deletion produces the dwarf *Pygmy* mutant in mice. In replication studies of adults sampled from across the height distribution, each copy of the C allele of the SNP was associated with an increase of 0.07 in the adult height z–score, which is equivalent to ~0.4 cm in height.

To determine the age at which the association appears, longitudinal data from the Avon Longitudinal Study of Parents and Children were analyzed. There was no evidence of association at birth, but strong association with height was observed at age 7 years, suggesting that the effect was on longitudinal skeletal growth. Since the *Pygmy* mice also displayed greatly reduced fat mass, the investigators sought evidence that the association affects BMI, but none was observed.

The authors discussed the fact that HMG proteins are DNA-binding proteins and often serve an architectural function with regard to chromatin structure and modeling, but they did not suggest possible mechanisms through which the polymorphism might alter bone growth.

Weedon MN, Lettre G, Freathy RM, et al. A common variant of *HMGA2* is associated with adult and childhood height in the general population. Nat Genet. 2007;39:1245-50.

First Editor's Comment: *It is ironic that although normal height is probably one of the most studied polygenic traits in humans, the first gene to show a strong effect in the general population is only now coming to the fore. It will be interesting to see how this story unfolds and what other genes are identified with new genomics analysis technology. The genetics of height variation assessed by linkage studies were reviewed in GGH.[1] These identified proteins, whose genes map to chromosomes 2q21 and 6q21 with locus interacting on an epistatic model, account for approximately 20% of height variation. These gene loci contain RUNX2 transcription factors with known functions on linear skeletal growth.*

William A. Horton, MD

Second Editor's Comment: *HMGA2 encodes "High Mobility Group AT-Hook 2" and is sited on chromosome 12q14.3. It is expressed in undifferentiated mesenchyme. HMG proteins alter chromatin configuration and thereby gene expression. They do so by the binding of their "AT hook domains" to AT-rich DNA; this alters conformation of the double helix and permits transcription complexes to either promote or inhibit transcription of targeted genes. Microdeletions or mutations of HMGA2 have been associated with benign neoplasia (lipoma, salivary adenoma, uterine leiomyoma). Truncation of HMGA2 secondary to a pericentric inversion of chromosome 12 with breakpoints at 12p11.22-12q14.3 has been associated with a syndrome of somatic overgrowth, advanced bone and dental ages, multiple lipomas and a cerebellar tumor.*[2] *Truncations of mouse ortholog Hmga2 (Hmg1c) result in somatic overgrowth, lipomas, and increase in body fat.*[3] *Homozygous deletion of mouse Hmga2 results in decrease in growth.*[4]

Allen W. Root, MD

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Height and Health-related Quality of Life

Findings regarding associations between height and psychosocial variables are inconsistent. To address perceived methodological and design weaknesses in previous studies, Christensen and colleagues sought to clarify the nature of this relationship by analyzing data collected through a national health survey. Their primary aim was to assess the relationship between stature and health-related quality of life (HRQoL) in an adult general population sample in the UK. Secondarily, they sought to evaluate potential moderating effects of social status, age, gender, and chronic conditions on the relationship between height and HRQoL.

This report is based on secondary analyses of the 2003 Health Survey for England (HSE03), conducted between January 2003 and March 2004, by the UK Department of Health. The HSE03 comprises a random general population sample for those living in private households in England (73% participation rate). Observations for 14,416 adults (>18 years of age) were included in the analyses. Height and weight were measured by a nurse; HRQoL was measured using the EQ-5D questionnaire (EuroQoL). The EQ-5D self-report consists of 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 3 possible levels reflecting no health problems, moderate health problems, and extreme health problems. Using a specific British EQ-5D scoring algorithm which converts total scores to quality adjusted life years, the 5 dimensions were summarized into a single score. An individual who has no problems in any domain scores 1.0 and death equals 0.0.

Mean EQ-5D scores were lower in the shorter compared with taller subjects, as well as being lower than the overall population mean. Based on statistical criteria, the total sample was split into 3 standardized height (HSDS) subgroups: (1) HSDS $\leq$–2.0, n=606; (2) –2.0 >HSDS $\leq$0, n=6580; and (3) HSDS >0, n=4760. In regression analyses adjusting for potential demographic confounds (age, gender, chronic illness, social class, and body weight), subgroup 1 had significantly lower EQ-5D scores compared with subgroups 2 and 3, and subgroup 2 received lower scores that subgroup 3. Based on regression coefficients, an increase of 1 HSDS would be associated with a statistically significant increase in the EQ-5D score of 0.061 for subjects $\leq$–2.0 HSDS, 0.010 for those between –2.0 and 0 HSDS, and 0.002 for those >0 HSDS. The increase in EQ-5D score with increasing height in the >0 HSDS, although statistically significant, was not considered of clinical significance. The main contributors to the reduced HRQoL in the shortest subgroup were problems with mobility, usual activities, and pain/discomfort. The authors concluded that increasing final height in children with short stature may be beneficial and could enhance HRQoL outcomes barring troublesome side effects and excessive cost of treatments.

Christensen TL, Djurhuus CB, Clayton P, Christiansen JS. An evaluation of the relationship between adult height and health-related quality of life in the general UK population. Clin Endocrinol (Oxf). 2007;67:407-12.

First Editor's Comment: *HRQoL should (1) represent a multidimensional construct, including several core dimensions (eg, physical functioning and symptoms, psychological and emotional state, and social functioning), (2) be patient, rather than physician, centered, and (3) reflect subjective evaluations of daily functioning and psychological well-being.*[1] *The use of patient reported outcomes, such as HRQoL measures, are encouraged and may soon be mandated by the FDA for the evaluation and approval of new drugs and medical interventions.*[2] *Rigorous standards for the development and psychometric evaluation of HRQoL measures have been promoted by the World Health Organization. It is therefore a positive development to see research published examining the relationship between measured height and subjective reports of QoL. In the FDA's review of growth hormone (GH) treatment for the indication of idiopathic short stature (ISS), HRQoL was <u>not</u> utilized as an endpoint in the approval process.*[3, 4]

Christensen and colleagues acknowledged that

inferring a causal relationship between height and HRQoL is not possible because of the single point, cross-sectional design of this survey. This limitation notwithstanding, they stated that their study "conclusively show(s) a significant correlation between adult height and HRQoL, which may indicate that improving final height in children with growth disorders who are receiving GH treatment should result in positive HRQoL outcomes, even if studies to date do not always show a benefit in childhood or adolescence." However, the use of a single method (the EQ-5D) makes such a statement (even as speculation) premature. Further, no controlled study, to date, has demonstrated a psychological benefit of increased growth velocity/height through the use of GH treatment.

A curious aspect of this study's findings concerns the pattern of scores for individual EQ-5D dimensions. Based on the authors' review of earlier studies suggesting that short stature exerts a psychosocial stress associated with poorer intellectual, psychosocial, and psychiatric function, it is surprising that the main contributors to reduced HRQoL in the shortest subgroup were problems with mobility, usual activities, and pain/discomfort. It is not obvious why shorter participants would more likely experience problems with walking or be confined to bed (as defined by the instrument), or experience more pain or discomfort unless, however, the short stature was accompanied by other features which compromised function—in which case, it is likely that the features accompanying the short stature, rather than the short stature itself, account for the compromised function.

Finally, the investigators pointed out the associations between height and HRQoL in adulthood are nonlinear; ie, it was only among the shortest survey participants (ie, <–2.0 HSDS) that meaningful improvements in HRQoL with increased height was predicted. Provided we accept correlational findings as evidence of causation, one implication of this is that GH-induced increases in adult height beyond –2.0 HSDS would not yield personal benefit. This finding therefore provides empirical support for the ethical argument of terminating GH treatment at the point at which the individual achieves an adult height within the lower portions of the normative range.[5]

In this context, it is noteworthy that this article is not accompanied by a disclosure statement indicating conflict; the first 2 authors' affiliation is listed as Global Development, Novo Nordisk A/S.

David E. Sandberg, PhD

Second Editor's Comment: *Projects which have tried to assess the effect of height on QoL, either in childhood or adult life, have been bedeviled by underpowered studies, the fallibility of questionnaires as a technique of QoL assessment, and the apparent extraordinary ability of children to adapt to their physical and environmental circumstances. The analysis of the data of Christensen et al showed a significant correlation between adult short stature and HRQoL. Height had a 6-fold greater correlation with HRQoL in the short adult population (ie, height <–2 SD) compared to the taller population subgroup. Very short subjects (height <–3 SD) were particularly affected and had very low QoL. It is likely that this study will be quoted in order to justify treatment of short children with GH. It should be appreciated that an improvement of adult height from –2.0 to –1.0 SD during GH therapy did not change the HRQoL to a large extent. Nevertheless when GH therapy offers the opportunity to make a large difference in adult height, for example in GH deficiency, adult HRQoL is likely to improve significantly.*

Martin O. Savage, MD

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Effects of Gluten-free Diet in Atypical Celiac Disease

Celiac disease frequently presents growth impairments as evidenced by an inflammatory enteropathy from T-cell hypersensitivity to certain cereal antigens; catch-up growth may be induced by initiation of a gluten-free diet. Street et al sought to study children longitudinally over their first year on the diet. Children with atypical celiac disease (patients with typical gastrointestinal symptoms as well as in an atypical fashion) were followed; outcome measures included changes and correlations in growth parameters, insulin-like growth factor (IGF) axis members, and the proinflammatory cytokines implicated in celiac disease pathophysiology, interleukin (IL)-6 and tumor necrosis factor (TNF)-α.

Twenty children (9 male), aged 4.2 to 14.2 (mean 9.6) years at diagnosis of atypical celiac disease, were followed; 17 completed the one-year evaluations and 3 were lost to follow-up. Of note, all had atypical celiac disease and presented with recurrent abdominal pain, anemia, nausea, occasional vomiting, and fatigue, or were screened due to family history. None had diarrhea or malnutrition, 11 children were prepubertal at diagnosis, and during the year's follow-up, 2 boys progressed from

Summarized results (mean + SEM).

Parameter	Controls	Celiac disease		P value <0.05: C= vs ctls D= pre/post diet N= neither
		Baseline	1 year on diet	
Height z–score	0.09 ± 0.3	0.51 ± 0.3	0.88 ± 0.4	D
Target height z–score	-0.4 ± 0.5	-0.4 ± 0.3	-	-
BMI z–score	0.5 ± 0.3	-1.6 ± 0.1	0.89 ± 0.3	C, D
IGF-I (ng/ml)	392 ± 47	208 ± 32	305 ± 35	C, D
IGF-II (ng/ml)	1098 ± 255	952 ± 52	1008 ± 119	N
IGFBP-1 (ng/ml)	61 ± 7	54 ± 8	45 ± 9	N
IGFBP-2 (ng/ml)	306 ± 35	493 ± 41	388 ± 74	C, D
IGFBP-3 (ng/ml)	4216 ± 286	4087 ± 300	4108 ± 281	N
IL-6 (pg/ml)	1 ± 0.1	2 ± 0.6	2 ± 0.7	C
TNF-α(ng/ml)	4 ± 1	2 ± 1	2 ± 0.4	C

Tanner 2 to 3. Mean bone age at diagnosis was 9.4 years, SEM = 0.9 years. Eighteen healthy children (5 male), aged 5.6 to 14.6 (mean 11.1) years, matched for pubertal stage, were evaluated as controls at baseline (Table). Their bone age data were not available.

The authors further examined various IGF/IGF binding protein (IGFBP) molar ratios, simple linear regression analyses and step-wise linear regression analyses to find additional correlates with baseline and treatment values. They concluded, "the data from this study confirm changes in the IGF and cytokine systems at diagnosis of celiac disease which tend to normalize on the gluten-free diet."

Street ME, Volta C, Ziveri MA, et al. Changes and relationships of IGFs and IGFBPs and cytokines in celiac disease at diagnosis and on gluten-free diet. Clin Endocrinol. 2008;68:22-8.

Editor's Comment: *The discussion section contains many conjectures about the mechanistic links between cytokines, IGF axis, growth, and disease of patients with celiac disease, stretching even to the increased risk of malignancy in patients with long-standing untreated celiac disease. All the data in this paper were associative. Associations are never sufficient to prove causation, as directionality and confounders remain unknown. There were no supporting mechanistic studies. Likewise, the paper measured serum concentrations. Local (ie, intestinal) concentrations of the cytokines and IGF axis members are more pertinent to disease activity, and changes may not be reflected in the serum levels. Finally, the study's ability to generalize is limited. The subjects all had atypical celiac disease, so the results do not necessarily support conclusions about celiac disease in toto. However, it is this very limitation that makes the findings of this paper intriguing. None of the patients studied had diarrhea, signs of malnutrition or history of celiac crisis. Although the BMI at baseline was significantly lowered, it was still within the normal range. Likewise, the gluten-free diet improved the height z–score, which was already normal, and even better than target height, at baseline. The finding of significant alterations in serum cytokines, IGF-I and IGFBP-2 within this population speaks to the sensitivity of the IGF system to this disease process.*

Adda Grimberg, MD

Levothyroxine Therapy on Ventricular Function in Neonates with Congenital Hypothyroidism

Decreased thyroid hormone levels are associated with poor left ventricular contractility and relaxation in hypothyroid adults. These abnormalities can be reversed by levothyroxine substitution therapy. Few studies have been done in neonates with congenital hypothyroidism and to date the results have been conflicting. Only standard echocardiography has been used to assess left ventricular function. Tissue Doppler echocardiography (TDE) is a new method that permits evaluation of regional and global left and right systolic and diastolic ventricular function and color codes the velocity of myocardial movement allowing for more accurate quantification.

Fifty neonates (17 to 28 days of age) who were full term and diagnosed with congenital hypothyroidism (TSH >5.6 mlu/L) with a depressed serum free thyroxine (FT_4 <10 pmol/L) or total thyroxine (TT_4 >54 mmd/L) were studied. A control group of 35 healthy neonates with normal thyroid function levels matched for age, sex, body surface area, and BMI were studied. None of the subjects had congenital heart disease as assessed by clinical and routine echocardiographic studies. Each neonate was studied with both conventional M-mode pulsed wave Doppler and with TDE. The infants were sedated with oral chloral hydrate for the studies. M-mode echocardiography measured left atrial aortic diameter, left atrial/aortic ratio, left ventricular fractional shortening, and left ventricular ejection fraction. In addition diastolic mitral and tricuspid inflow velocity was measured. The TDE permitted measurement of peak early diastolic mitral annular velocity, peak late diastolic mitral annular

velocity, and peak systolic mitral annular velocity, as well as similar measurements for tricuspid velocity.

Using conventional Doppler echocardiography, markers of left ventricular systolic global function were significantly lower in the infants with congential hypothyroidism. In addition, early and late mitral and tricuspid valve diastolic function were significantly lower in the infants with congential hypothyroidism. After a month of levothyroxine (L-T_4) therapy several of the left ventricular parameters improved, but left atrial and aortic diameter did not change. Significantly reduced mitral systolic and early diastolic velocity was found by TDE in the group with congenital hypothyroidism. These significantly increased after therapy, while the peak annular mitral and tricuspid velocity remained unchanged.

Mao et al pointed out that their study was the first comprehensive report of systolic and diastolic function of both ventricles in neonates with congenital hypothyroidism and their data showed impaired left ventricular systolic function which normalized with L-T_4 therapy. Their data also showed that infants with congenital hypothyroidism do not have abnormal left atrial structure. The use of the TDE confirms subclinical impairment of both left and right ventricular contractile function in neonates with congenital hypothyroidism, as well as diastolic dysfunction of both ventricles. The authors concluded that their data underscore the importance of early detection and treatment of infants with hypothyroidism.

Mao S, Wang Y, Jiang G, Zhao Z. Effects of levothyroxine therapy on left and right ventricular function in neonates with congenital hypothyroidism: a tissue Doppler echocardiography study. Eur J Pediatr. 2007;166:1261-5.

Editor's Comment: *This is a very interesting and comprehensive study which shows convincing evidence that there is significant cardiac dysfunction in neonates with congenital hypothyroidism. The presence of a control group adds to the significance of the findings. It is interesting that this study, conducted in China, was performed on infants aged 17 to 28 days, prior to the initiation of L-thyroxine therapy. Details of screening for congenital hypothyroidism in China were not presented. It is disturbing that treatment of a hypothyroid infant would be delayed as long as 28 days. One would hope that with improvement in screening techniques such a delay could be reduced. Clearly the authors have presented significant information demonstrating the need for early identification and treatment of this disorder.*

William L. Clarke, MD

Uterine Development in Turner Syndrome

Bakalov and associates performed a cross-sectional study evaluating uterine development in 86 women with Turner syndrome (TS), aged 18 to 45 years, who were participating in a comprehensive NIH study. All subjects had a karyotype by G-banding consistent with TS in at least 70% of 50 white blood cells. The women were evaluated by either transabdominal (n=68) and/or by transvaginal (n=20) ultrasonography. Longitudinal and anterior posterior fundal diameters were calculated as well as the maximal transverse uterine diameter. Normative data were used to characterize uterine maturity. Historical and treatment data including pubertal development, age of initiation of hormone replacement therapy, type of estrogen used, years of estrogen use, and history of growth hormone therapy were recorded. In the case of spontaneous menarche, the time interval from menarche to the development of amenorrhea was noted.

The mean age of the study population was 31.8 ± 7.3 years. Most subjects (93%) had a karyotype consistent with TS, while 6 (7%) had mosaicism. None had a Y chromosome (intact or abnormal), 15% had spontaneous menarche at age 12.2 ± 1.7 years, but had developed amenorrhea by their late teens. All other subjects (73/86) had started estrogen at an average age of 15.7 ± 4.1 years. Thirty percent (26/86) had also been treated with growth hormone. Almost one quarter (24.4%; 21/86) had a fully developed uterus both in size and shape, while many (44%; 36/86) had a smaller size uterus (transitional) and 31.4% (27/86) had an immature (cylindrical shaped) uterus. Regression analysis demonstrated that uterine size was influenced significantly by age, years of estrogen use, current use of hormone replacement therapy, history of spontaneous menarche and the type of estrogen medication. There was no correlation between age of first exposure to estrogens and the size of the uterus. The degree of uterine maturity was positively associated with years of estrogen use, history of spontaneous menarche, and negatively associated with the lack of current hormone replacement therapy.

The authors reviewed recent studies from Germany[1] which showed that only mosaic females develop normal uterine size and that karyotype was the only significant predictor of normal uterine development. Findings in the current study were significantly different and 57% of the subjects with a mature uterus had a 45,X karyotype. This may be explained by an average longer duration of estrogen exposure. The authors stated that these findings are encouraging for those women with TS who wish to carry a successful pregnancy. A recent review of women with TS in the US participating in oocyte donation programs found that 69% became pregnant and these pregnancies resulted in the birth of a live infant.[2]

Bakalov VK, Shawker T, Ceniceros I, Bondy CA. Uterine development in Turner syndrome. J Pediatr. 2007;151:528-31.

Editor's Comment: *These authors presented some truly encouraging information for endocrinologists to share with their patients with TS. Indeed hormone replacement therapy is associated with normal uterine development while the age of starting hormone replacement therapy is not a critical factor. Thus those women with TS who wish to participate in oocyte donation programs should be encouraged to do so or may be encouraged to do so with reasonably good assurance that their uterus should be capable of sustaining a normal pregnancy. As the authors noted, their study could have unexpected biases due to its cross-sectional nature.*

William L. Clarke, MD

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GH Treatment Effects on Body Composition in SGA

The use of growth hormone (GH) therapy in small for gestational age (SGA) children with short stature, now approved and licensed both in the US and Europe, requires critical appraisal. Body composition in childhood may be affected by alteration of fetal growth. SGA infants who show catch-up growth tend to become obese and may be at risk for metabolic syndrome in adult life. However, SGA children who remain short are thin and have a low BMI and possibly compromised bone mineral density. The group of 25 SGA subjects (birth weight and current height <–2 SD) reported in this study were prepubertal and randomized to receive either GH therapy (n=16) or act as untreated controls for 3 years and then start GH therapy (n=9). Heights in both groups were <–2 SD and the daily GH dose was 1 mg/m^2 body surface area.

Clinical characteristics were comparable in the 2 groups. In the untreated subjects lean body mass (LBM) decreased during the 3 years (P<0.01) contrasting with the GH-treated group which showed catch-up increase of LBM. When the untreated subjects started GH, their LBM SDS also increased significantly. Therefore GH therapy, in the dose described, induced catch-up of LBM. However percentage body fat decreased in the GH-treated subjects. Bone mineral density SDS measured by DEXA increased significantly in the GH-treated group compared to the untreated subjects.

Willemsen RH, Arends NJ, Bakker-van Waarde WM, et al. Long-term effects of growth hormone (GH) treatment on body composition and bone mineral density in short children born small-for-gestational-age: six-year follow-up of a randomized controlled GH trial. Clin Endocrinol (Oxf). 2007;67:485-92.

Editor's Comment: *These findings are of interest, but their clinical relevance remains uncertain. The anabolic effects of GH on muscle bulk and bone mineralization are demonstrated, as is its lipolytic effect. However the benefit to the child of these changes is difficult to assess. Is the improvement in BMD really going to prevent development of osteoporosis and increased fracture risk in adult life? The answers are unknown. Is the reduced LBM in the untreated short SGA child actually a disadvantage to the child? Again we are not certain. However, in this report the carefully studied longitudinal changes in body composition which occur during GH therapy are useful in documenting the anabolic and lipolytic effects of GH in short SGA children.*

Martin O. Savage, MD

Widespread Monoallelic Expression of Human Autosomal Genes

With certain exceptions, it is generally assumed that maternally and paternally-derived copies (alleles) of each gene are expressed at comparable levels in humans. The first exception is inactivation of most of the genes residing on the X-chromosome in females—so called X-inactivation. Half of the cells in an embryo on average randomly inactivate the paternal X chromosome and half inactivate the maternal X chromosome around the time of implantation. The second exception involves imprinting of autosomal genes, such as IGF-2, on a parent-of-origin basis. A third exception is a small group of autosomal genes that are subject to random monoallelic expression; these include genes encoding odorant receptors, T cell receptors, interleukins, and natural killer cell receptors. There is new evidence that monoallelic expression of autosomal genes may be much more extensive than previously believed.

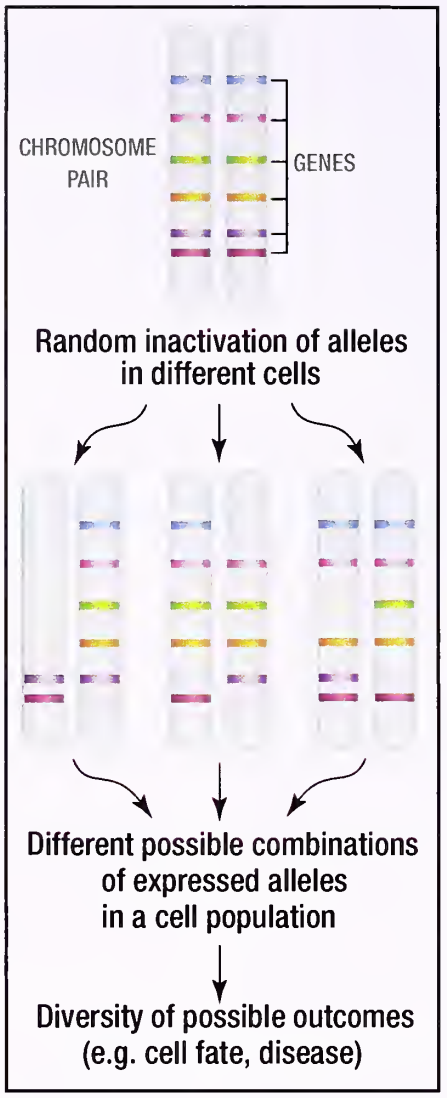
Gimelbrant et al exploited the growing number of single nucleotide polymorphisms (SNPs) and advances in gene chip (array) technology to survey allele-specific transcription of about 4,000 genes in lymphoblastoid cell lines from 3 individuals. They took advantage of the observation that once a cell decides to express one of 2 alleles, the clonal descendents of this cell continue to express the selected allele. Since lymphoblastoid cells are polyclonal, they were able to derive clonal B cell lines using single-cell cloning.

To perform the genome-wide screen for monoallelic transcription, the investigators developed protocols to distinguish polymorphic allele expression based on detection of SNPs in nuclear RNA, which is enriched in intronic RNA, where most SNPs associated

with genes reside. Conversion of this RNA to double-stranded cDNA and analysis on a SNP array generated "transcriptosome-derived genotypes" that allowed monoallelic expression to be identified. Filters were used to minimize cDNA genotyping artifacts. About 10% of SNPs were reliably called from this analysis, which was expected since most of the other SNPs are likely present in regions of the genome that are not expressed by B cell lines that were studied.

As proof-of-concept, the investigators first showed that random inactivation of X-chromosome genes could be detected in the clonal cell lines and then demonstrated as an example of their approach that monoallelic expression of the amyloid precursor protein gene could be detected. They next turned to genome-wide screening.

On the array used for analysis, there were SNPs present for ~11,000 genes. They were able to detect allele-specific transcription for ~4,000 genes in 2 or more cell clones. Of the ~4,000 genes examined, 2.2% were detected as monoallelically expressed with multiple informative SNPs per gene per clone. An additional 7.3% of assessed genes were identified as monoallelically expressed based on a single informative SNP per gene per clone. The genes included both B cell-specific genes and ubiquitously expressed genes. The investigators suggested a conservative estimate that over 1,000 genes are subject to random monoallelic expression in humans.



Generating diversity. Alleles are randomly inactivated on a pair of chromosomes in a human somatic cell. The various patterns of inactivation in progeny cells are then stabilized (epigenetically). This can generate diverse cellular and physiological outcomes. Reprinted with permission Ohlsson R. Science. 2007;318:1077-8. Copyright © 2007 AAAS. All rights reserved.

Several interesting observations were made. For example, the choice of expressed allele was made independently for each gene within a given clonal cell line. This is in contrast to the chromosomal-wide coordination characteristic of X-inactivation. Another finding was that a disproportionately large fraction of genes coding for cell surface proteins—transmembrane receptors and surface proteins was detected.

The authors concluded by suggesting that at least 1,000 human genes display random monoallelic transcription that could contribute to genetic diversity within tissues of an individual as well as between individuals. A commentary by Ohlsson[1] notes that although monoallelic expression has been known in humans, this study by Gimelbrant expands the concept further especially by documenting it in a much larger number of genes than previously appreciated. He briefly discusses possible mechanisms that could account for the phenomenon as well as its potential role in modulating disease.

Gimelbrant A, Hutchinson JN, Thomson BR, Chess A. Widespread monoallelic expression of human autosomes. Science. 2007;318:1136-40.

Editor's Comment: *This is one of several publications in recent years that challenges what we were taught about mendelian genetics. Of note, several genes relevant to human growth disorders were identified as displaying monoallelic expression including the growth hormone receptor gene (GHR) and genes that harbor mutations responsible for Ellis van Creveld syndrome (EVC) and the trichorhinophalengeal syndrome 1 (TRPS1). It seems quite plausible that monoallelic expression of these genes could contribute to the clinical variability of these conditions.*

Lymphoblastoid cells have very different functions compared to chondrocytes, osteoblasts and other cells that contribute to skeletal growth; and their patterns of gene expression may differ dramatically. Screening the latter cells for monoallelic transcription would be technically much more difficult than for lymphoblastoid cells, but it would likely reveal monoallelic expression of additional growth related genes.

William A. Horton, MD

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Stroke, Cardiac Disease and Diabetes Mellitus in Hypopituitarism

The impact of long-term growth hormone deficiency (GHD) and of long-term growth hormone (GH) treatment on cerebrovascular and cardiovascular diseases and diabetes mellitus is unknown. Holmer et al evaluated the incidence of nonfatal stoke and cardiac events and the prevalence of type 2 diabetes mellitus (T2DM) in a cohort of GHD patients and healthy controls. The authors also studied the effects of cardioprotective drugs and 6

years of GH-replacement treatment in this population. The incidence of nonfatal stroke and cardiac events was estimated retrospectively from questionnaires in 750 GHD patients (53% males and 47% females) and in 2314 matched population controls. GHD patients were recruited from the departments of endocrinology at all Swedish University hospitals and one county hospital. All patients were diagnosed as having severe GHD by dynamic testing (peak GH <3 mcg/L). The lifelong incidence of nonfatal stroke was tripled in GHD women and doubled in GHD men. A decline was noted in both genders following the detection of the first pituitary hormone deficiency and GHD, a period of time during which most patients received GH therapy. The lifelong incidence of nonfatal cardiac events declined in GHD men; GHD women had a higher prevalence of T2DM. Women were twice as likely to be taking lipid-lowering drugs as the population controls, while GHD men had a 28% higher prevalence for the use of antihypertensive medication. The authors concluded that the decreased risk of nonfatal stroke in both genders and of nonfatal cardiac events in GHD men may be due to the larger prescription of cardioprotective drugs and to 6 years of GH-replacement. The increased prevalence of T2DM in GHD women can be partly attributed to a higher body mass and to decreased physical activity.

Holmer H, Svensson J, Rylander L, et al. Nonfatal stroke, cardiac disease and diabetes mellitus in hypopituitary patients on hormone replacement including growth hormone. J Clin Endocrinol Metab. 2007;92:3560-7.

Editor's Comment: *An increased incidence of cerebrovascular and cardiovascular mortality in patients with hypopituitarism on conventional hormone treatment, but without GH therapy, has been reported in recent epidemiological studies.[1,2] GHD is believed to be responsible for the early atherogenesis in hypopituitarism, as cardiovascular risk factors have been improved with GH treatment in this group of patients. Glucose intolerance, T2DM, and hypertension are increased in GHD.[3] Diastolic blood pressure tends to decrease with GH treatment, while insulin sensitivity is impaired following initial GH replacement, but may improve later as fat mass is reduced. This study showed that hypopituitary patients had a higher lifelong incidence of nonfatal stroke (triple in GHD women and double in GHD men), although cerebrovascular events decreased in men and women during the periods following the diagnosis and the treatment of the pituitary hormone deficiencies and of GHD. This decline was probably due to the long-term use of GH and the replacement of thyrosine and glucocorticoids. Additionally, patients may also benefit from the increased administration of lipid-lowering and antihypertensive medications. The increased prevalence of T2DM in GHD women could not be attributed to overtreatment with GH as the IGF-I level was at mid range. Additionally, acromegaly and Cushing's disease were excluded in these patients, thus the increased prevalence of T2DM was partly attributed to their higher BMI and their lower physical activity. Long- term surveillance for cardiovascular disease and T2DM seems necessary in hypopituitary patients; the institution of appropriate treatment with hormone replacement and cardioprotective drugs plays a positive role in decreasing the risk of nonfatal stroke and the risk of nonfatal cardiac events in men; an increased prevalence of T2DM seems to be present in GHD women.*

Roberto Lanes, MD

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Growth and Metabolism in In Vitro Fertilization Children

In vitro fertilization (IVF) singleton children have an increased risk of malformations and low birth weight. They also face an increased risk of disorders with overgrowth partly due to abnormal methylation patterns of imprinted genes. Nutritional manipulation early in fetal life has also been shown to reduce methylation and over expression of non imprinted genes. Miles et al conducted a study regarding the long-term outcome of IVF children, an area in which there is still a lack of information. The authors investigated growth and changes in the metabolic and hormonal profile of this population. Healthy prepubertal children aged 4 to 10 years, born at term, after singleton pregnancy, were recruited into IVF and control groups. All subjects had been breastfed. There were 69 IVF children (5.9 years) and 71 control children (6.9 years). Anthropometric measurements and BMI were recorded, focusing on fat and glucose metabolism, and insulin-like growth factor (IGF)-I levels. Both groups were matched for parental anthropometry, socio-economic factors and dietary conditions. IVF children were taller than controls (and girls were even more so) when height was corrected for parental height. This increase in stature was proportionate. It occurred despite a lower birth weight. The corrected BMI was lower in the IVF group and there was no difference in percent fat assessed by DEXA. There was a trend toward higher IGF-I levels in the IVF group with patients above 7 years of age having the highest levels. The IGF-I/IGF binding protein (IGFBP)-3 ratio was also increased. IGF-II was elevated as well in the IVF group without any age related effect.

For all children there was an association between tall stature and high IGF levels. A favorable metabolic profile was found in the IVF group with higher HDL, lower triglycerides and a low total to HDL/cholesterol

ratio. There was no difference in body composition. The authors speculated that IVF results in epigenetic changes altering genes involved in growth and metabolism that could be similar to the changes shown in specific syndromes like Beckwith-Wiedemann.

Miles HL, Hofman PL, Peek J. In vitro fertilization improves childhood growth and metabolism. J Clin Endocrinol Metab. 2007;92:3441-5.

Editor's Comment: *Since this technique was introduced, IVF has accounted for a growing number of births.[1] Only recently have follow-up studies focused on the postnatal outcome of these children. A few informative and elegant studies have already drawn our attention to epigenetic changes that induced major malformations. The most investigated area is the overgrowth disorder of Beckwith-Wiedemann with a variable clinical expression from the full syndrome to isolated overgrowth. It has been shown that there are imprinting disorders observed in humans and animals born after the use of assisted reproductive technology. The genomic imprinting defects relate to an epigenetic marking of certain genes, resulting in monoallelic expression in a parent-of-origin-dependent manner. Imprinting control elements are characterized by differentially methylated regions in which the imprinted allele is methylated and the other parental allele is unmethylated. Imprinting is established during the development of the germ cells and must be maintained at a critical stage of pre-implantation development when the rest of the genome is subjected to a wave of demethylation. These imprinted genes have a major role in fetal growth and development. All imprinting disorders observed after assisted reproductive technology involve the maternal side inducing a maternal to paternal switch, with activation of non-coding RNA on the maternal side. The cause of association with IVF is unknown. One can only suggest that nutritional and environmental factors, or periconceptional or preimplantation conditions could result in these alterations. Furthermore, we do not know whether the clinical changes already observed are reversible. Hence their significance remains unclear.*

Miles et al described an increased incidence of tall stature in prepubertal children which was accompanied by increased levels of IGF-I and IGF-II. It was suggested that this "overgrowth" might be the consequence of programmed endocrine changes related to the IVF process. Remarkably, in this group of children the body composition and the lipid profile were normal. However, in another recent study the offspring of those conceived by IVF presented significantly higher peripheral adipose tissue.[2] It is difficult to compare the metabolic status of the 2 populations because of age differences and methods used in both studies. Appropriate follow-up should be established for all IVF children.

Raphaël Rappaport, MD

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Hypopituitarism Following Traumatic Brain Injury and Subarachnoid Hemorrhage

The hypothalamus and pituitary are vulnerable to injury and dysfunction following traumatic brain trauma (TBI) and subarachnoid hemorrhage (SAH). These constitute worldwide public health problems and leading causes of death and disability in young adults. Survivors of both TBI and SAH are at a great risk of significant neuroendocrine dysfunction, adverse physical and/or psychological problems, depression, and sleep disturbances that result in disturbed quality of life (QOL).

Schneider et al searched the MEDLINE database for articles published between 2000 and 2007 pertaining to TBI and SAH. They identified 19 studies including 1137 patients (1015 TBI patients and SAH 122 patients). Only 2 of these studies (with 74 patients) reported on pediatric populations. The authors investigated 13 studies (with 809 TBI patients and 102 SAH patients) that were performed at least 5 months following the injury (chronic phase). They excluded studies in the early phase after injury to avoid the confounding effect of acute critical illness on neuroendocrine function and the pediatric populations for reasons of homogeneity. The pooled prevalence of anterior hypopituitarism in the chronic phase after TBI and SAH was 27.5% (95% CI, 22.8%-28.9%) and 47% (95% CI, 37.4%-56.8%), respectively. The pooled prevalence of hypopituitarism was greater in patients with severe TBI, as compared with those with mild or moderate TBI (as defined by the Glasgow Coma Scale). On the contrary, clinical severity of SAH did not help discriminate between patients at high and low risk of developing hypopituitarism. Early neuroendocrine abnormalities were transient in some patients while hypopituitarism evolved over time in others.

The authors considered that hypopituitarism appears to be a common occurrence following TBI and SAH and might contribute to morbidity and poor recovery after brain injury although most cases remained unrecognized and untreated. All patients hospitalized for TBI or SAH should be evaluated for endocrine alterations long-term.

Schneider HJ, Kreitschmann-Andermahr I, Ghigo E, Stalla GK, Agha A. Hypothalamopituitary dysfunction following traumatic brain injury and aneurismal subarachnoid hemorrhage. A systemic review. JAMA. 2007;26:1429-38.

Editor's Comment: *Another article regarding TBI published in the recent literature was reviewed in GGH last year.[1] The current paper by Schneider et al provides*

specific information regarding patients with SAH. Considering the large number of individuals who have TBI and SAH each year, post-traumatic hypopituitarism is an important public health issue. TBI and SAH pose substantial risks to hypothalamopituitary dysfunction. Hypopituitarism after TBI and SAH might contribute to a delayed or hampered recovery during rehabilitation. However, in both adults and children, a large number of patients with hypopituitarism after TBI or SAH remain undiagnosed and untreated.

Possible causes of hypopituitarism include hemorrhage, infarction, ischemia, necrosis, fibrosis, swelling, stalk transaction, or direct trauma to the hypothalamus, stalk, and/or pituitary region. The severity of TBI seems to be an important risk factor for developing hypopituitarism, however, post-traumatic hypopituitarism can also manifest after even mild TBI. Whereas hypothalamopituitary dysfunction occurred without regard to the severity of SAH.

The signs and symptoms associated with hypopituitarism are often nonspecific and mimic the sequelae of TBI and SAH such as depression, neuropsychological deficits, or personality changes. They are likely to be overlooked if endocrine dysfunction is not actively assessed. Moreover, hormonal deficits may contribute to the chronic disability and the physical, cognitive, health, and social sequelae in patients with TBI and SAH. Therefore, accurate endocrine evaluation and long-term follow-up of TBI and SAH patients are necessary in order to detect the occurrence of hypopituitarism, regardless of clinical evidence for hypothalamopituitary dysfunction. In order to improve outcome and quality of life of TBI and SAH patients, adequate hormone replacement therapy may be necessary in those who develop hypopituitarism. It is necessary for physicians as well as patients and family members to know that hypothalamopituitary dysfunction following TBI and SAH may occur long after the initial trauma. A close collaboration among neurosurgeons, neurologists, rehabilitation specialists, internists, pediatricians, and endocrinologists is essential to achieve a coordinated approach to the care of patients with TBI and SAH. The consensus guidelines for assessment and for clinical practice of such patients have been published.[2,3]

Yoshikazu Nishi, MD

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FTO Gene Association with BMI and Obesity

Frayling et al and Dina et al have both linked a common variant in a set of single nucleotide polymorphisms (SNPs) in the first intron of FTO (fat mass and obesity associated gene; OMIM 610966, chromosome 16q12.2) with early onset of severe obesity in children and adults of European ancestry. FTO has 9 exons; its product and function are as yet unknown. In the report of Frayling et al, a genome-wide association study of 490,032 SNPs and their relationship to type 2 diabetes mellitus (T2DM) was conducted and 10 SNPs in intron 1 of FTO (designated A allele) was found to be closely related to this disorder. Further analysis revealed an even stronger association between BMI and the FTO intron 1 SNPs variant. In adults of all ages and both genders, each A allele was associated with an increase in BMI of 0.10 z–score units (~0.4 kg/m^2). Adult carriers of one A allele had an odds ratio of 1.31 for being overweight (BMI >25 kg/m^2) and of 1.18 for being obese (>30 kg/m^2); subjects homozygous for the A allele had 1.38 risk of being overweight and a 1.67 risk of obesity. Similar studies in children and adolescents between 7 to 14 years of age revealed that those with one A allele had an odds ratio of 1.27 for being overweight and of 1.35 for being obese. Waist circumference, skin-fold thickness, and DEXA measurement of fat mass were increased in children with the A allele. Frayling and co-workers found no functional variants in the exonic sequences of FTO relative to the SNPs variation in intron 1. Thus, the manner in which this variant of FTO affects weight accumulation is as yet unknown. Dina et al also associated the A allele with severe obesity in adults (BMI >40 kg/m^2) as well as with early-onset obesity in children, but found no mutations in the coding regions of FTO. Both groups concluded that a variant in SNPs in intron 1 of FTO is associated with an increased risk of obesity in children and adults, but the mechanism of the effect remains unexplained at present.

Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007;316:889-94.

Dina C, Meyre D, Gallina S, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet. 2007;39:724-6.

Editor's Comment: *Experimentally in mice, deletion of the chromosome segment in which FTO is located is embryonically lethal in the homozygotic animal and is marked by fused toes and thymic hyperplasia in the heterozygotic mouse that is of normal weight. Therefore, the composition, structure, and functional properties of the product(s) of FTO variants may need to be identified by methods other than those that attenuate (or enhance?) expression of FTO in experimental animals. If these goals can be successfully accomplished and the*

functional relationships between variants of FTO and the regulation of energy metabolism and conservation elucidated, then it may be possible to design agents that can be directed to sites of FTO action that will ultimately lead to improved methods of weight control. Interestingly, the presence or absence of the A allele was not associated with birth weight.

Allen W. Root, MD

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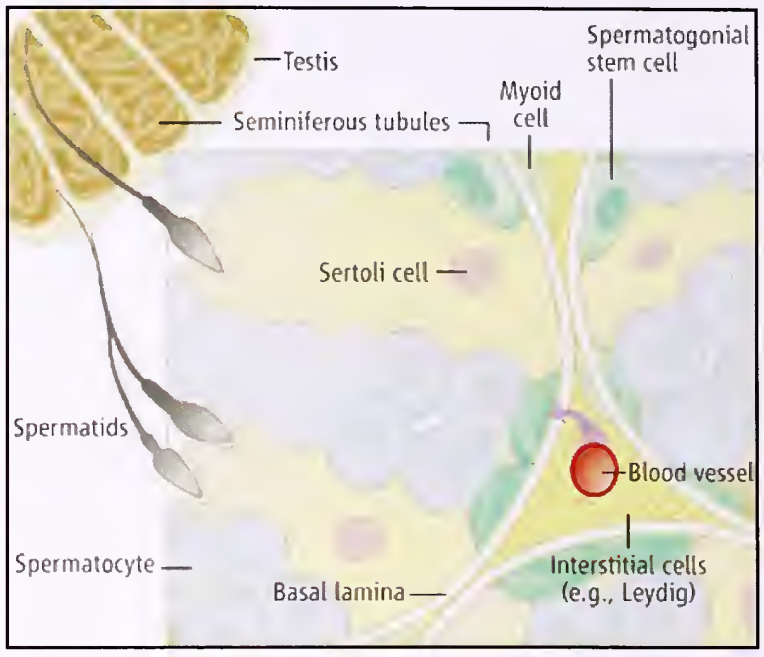
A Niche for Undifferentiated Spermatogonia

In human males, spermatogenesis proceeds over several decades. Scattered throughout the spermatogenic tubules of mammalian testes are spermatogenic stem cells (cells that are able to self-renew and to differentiate into cells with more specialized functions) that appear to be localized to specific regions within the tubule (Figure). In mice, undifferentiated spermatogenic stem cells constitute less than 1% of testicular cells and periodically differentiate into primitive type A single (As) spermatogonia that then give rise to daughter cells—A paired (Apr) and A aligned (Aal)—chains of 4 to 32 cells—that in turn evolve into more mature spermatogenic cells.[1] The tubular regions that harbor the most primitive and undifferentiated spermatogenic stem cells are termed "niches" and are deemed important because of the environment provided therein that enables the undifferentiated A cells to survive and from which daughter cells migrate and populate the spermatogenic tubules permitting the decades-long process of spermatogenesis. Yoshida and co-workers have identified the sites of As localization by labeling undifferentiated A cells with green fluorescent protein (GFP) expressed in response to a regulatory sequence of a gene (Ngn3) expressed in spermatogenic cells. Utilizing time-lapse imaging to follow the course of GFP cellular expression in intact mouse testes, they localized the earliest mouse spermatogenic stem cells (As) to specific regions in spermatogenic tubules; these cells reside in a basal tubular compartment adjacent to the interstitium and across from blood vessels that are surrounded by interstitial cells (including Leydig cells); these sites are characterized by turns in the spermatogenic tubule and by branching of their associated blood vessels. As As cells transitioned to Apr and Aal cells, they migrated from the site of origin and spread throughout the basal tubular compartment giving rise to more differentiated spermatogonia, spermatocytes, spermatids, and sperm. The investigators confirmed these observations by transplantation of testicular fragments from donor testes that had been cleansed of vessels and interstitium to sites beneath the tunica albuginea of recipient testes in vivo. Three months later, the grafts had revascularized, the interstitium had been reconstituted, and spermatogenesis was normal; As cells were again localized to turns in the tubules across from branch points of the blood vessels that were themselves encased in interstitial cells. The authors suggested that the niche for As cells by proximity of the tubular basal compartment to the branch point of blood vessels and to abundant interstitial cells provides



At home, in small narrow places. Spermatogonial stem cells localize to interstitial regions between seminiferous tubules in the mouse testis. This implies that interstitial cells and branching blood vessels secrete factors (arrow) that influence stem cell fate.
Credit: Adapted by P. Huey/Science, Reprinted with permission DiNardo S, Braun R. Science.2007;317:1696-7. Copyright © AAAS 2007. All rights reserved.

a microenvironment in which "signals" from these cells recruit, nourish, and stimulate differentiation of spermatogenic stem cells. The biochemical nature of these signals is unknown but likely include testosterone, a factor known to be important for the earliest stages of spermatogonial differentiation, as well as products of the Sertoli cells. That niches can be reconstituted (as demonstrated by the testicular graft experiments) indicates that new niches can be developed, a process that would support long-term spermatogenesis.

Yoshida S, Sukeno M, Nabeshima Y-I. A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis. Science. 2007;317:1722-6.

Editor's Comment: *Identification of the sites within the spermatogenic tubule that harbor undifferentiated spermatogenic stem cells may prove beneficial in isolating such cells. Inasmuch as these are cells with the diploid number of chromosomes (ie, prior to the first meiotic division), spermatogenic stem cells may ultimately provide a source of pluripotential stem cells.*[1]

Allen W. Root, MD

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In This Issue
Reviews & Comments

GROWTH HORMONE ADMINISTRATION: IS IT SAFE AND EFFECTIVE FOR BODYBUILDING AND IMPROVED ATHLETIC PERFORMANCE?

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INTRODUCTION

Athletes and the media have demonstrated great interest in the subject of administration of growth hormone (GH), popularly referred to as *doping*. Thus, a review of the evidence for safety and efficacy in athletes, especially, adolescents, is warranted. Many other drugs are administered off-label, particularly a majority given to children and adolescents. However, recombinant human GH (rhGH) is unusual because off-label prescribing and administration is illegal if given for indications not approved by the US Secretary of Health and Human Services (HHS). In this article—following short sections on the physiology of GH and the clinical role of rhGH—the data pertinent to athletic performance and problems with detection of doping are presented. This article ends with the specific legal issues and the activity surrounding further legal action with reference to rhGH and athletes.

The consideration of rhGH as an ergogenic aid and its potential to enhance athletic performance and/or body composition goes back many decades.[1] Due to the banning of rhGH use for officially sanctioned sport, there has been a large effort to detect its

From The Editor's Desk

Dear Colleague:

We have applied for and expect to be granted the opportunity of granting CME credits for reading GGH. Thus, while you enjoy the lead article and each one of the reviews you may also meet your requirements for licensure in the comfort of your home or office. In the near future the CME application and questionnaire will be posted on line. Once you complete it you will attain the necessary credits. Hopefully this feature will add to the value you place to the journal.

The current issue of GGH includes a very timely review of the safety and effectiveness of growth hormone for body building and improved athletic performance. The article by Dr. Alan Rogol brings us up to date and clarifies the issues that were widely discussed during this past summer's Olympic Games in China. However the article should also serve the pediatric endocrinologist to guide their patients and their families who seek this treatment to enhance their children's abilities. It should also serve as a resource to warn them of the illicit use of this product for such purposes, as well as to caution them to avoid falling prey to the multiple ineffective, expensive, and unregulated products available for purchase through the Internet. I also want to bring to your attention the reviews on the genetics of stature and the genetics of dwarfism. These excellent reviews include a synthesis of the state of the art of the most current papers and concepts in the field. Also noteworthy is the review dealing with the limited workforce of pediatric endocrinologists.

The economic situation of GGH continues to worsen with the downturn of the economy, yet we do not qualify for a bailout. Therefore I would appreciate your support in the form of a generous contribution so we may continue fulfilling your educational needs. I am sure you are being swamped with donation requests, please put GGH on top of your list and make your tax-deductible contribution to Pediatric Sunshine Academics, Inc. at www.PedSacademics.org or mail to 1040 Alston Road, Santa Barbara, CA 93108.

Happy Holidays and Best Wishes for 2009
Fima Lifshitz, MD
Editor-in-Chief

GGH is supported by unrestricted medical education grants from EMD Serono and Pediatric Sunshine Academics, Inc.

presence in athletes.[2,3] More than ten years and millions of dollars have been spent on devising and implementing tests to detect doping. Despite anecdotal reports of the widespread use of doping no athlete has been sanctioned for the use of rhGH, even with the multiple seizures of rhGH from athletes and teams.

Why should pediatric endocrinologists be concerned about this seemingly esoteric subject? Pediatric endocrinologists have been counseling children, adolescents, and their parents about the height-increasing properties of rhGH for decades. The use of rhGH is legitimate in children who are truly small, such as those with GH deficiency, and other disorders (Table 1). As athletics and sports play an ever increasing and important role in the lives of children and adolescents, parents seek a competitive edge for their children. Families spend thousands of dollars on coaching and equipment in hopes of the possibilities of college scholarships and/or professional contracts. Therefore, pediatricians are now being asked to prescribe rhGH because of parental "beliefs" that it will improve athletic performance in children and adolescents. Although very expensive, parents may consider rhGH as seemingly little different from very expensive coaches, equipment, and training camps.

Table 1. FDA-approved indications for rhGH therapy in children

Growth hormone deficiency
Chronic kidney disease
Turner syndrome
Small-for-gestational age infants who fail to catch-up to the normal growth percentiles
Prader-Willi syndrome
Idiopathic short stature
SHOX gene haploinsufficiency
Noonan syndrome

PHYSIOLOGICAL ROLE OF GH

The physiological role of GH is to increase linear growth in children, to promote anabolic (tissue building) metabolism, and to alter body composition as part of this anabolic role. Growth hormone actions include the hepatic and local synthesis and release of its main mediator-protein, insulin-like growth factor (IGF)-I. The growth-promoting effects of GH include longitudinal bone growth by actions at the epiphysis and the differentiation of the prechondrocytes. GH shares some of these roles with IGF-I, meaning that the direct effect of GH and/or local production of IGF-I are both necessary for optimal growth.[4]

Stimuli to GH release include deep sleep, exercise, stress—including heat stress—hypoglycemia, and some amino acids. Some pharmacological agents are also stimuli to the release of GH, for example, beta-2 adrenergic agonists, clonidine, L-DOPA, and estrogens and androgens (through an estrogen dependent mechanism). Inhibitory influences include obesity or ingesting a carbohydrate-rich diet. The direct effects of GH lead to increased glucose availability, increased free fatty acid levels and an increase in amino acid uptake by muscle. Longer term effects are mediated via IGF-I and include endocrine and paracrine effects in muscle and bone.[4]

Alterations in GH-deficient subjects include: the reduction of lean body mass, an increase in body fat, and a reduction in bone mineral density. From this the major metabolic effects of GH can be deduced. Administering rhGH reverses many of these alterations. However, it is not quite so simple, because GH has different effects depending upon the time following natural secretion (GH) or exogenous administration (rhGH). It is insulin-like in the first few minutes, but after several hours GH becomes diabetogenic and is anti-insulin at the liver and at peripheral sites, glucose utilization is decreased, lipolysis is increased, and the tissues are refractory to the acute insulin-like effects for several hours. The direct actions of GH include amino acid transport in muscle permitting protein synthesis and an increase in nitrogen balance, increased fat mobilization through lipolysis (increased triglyceride hydrolysis to free fatty acids and glycerol and reduction in fatty acid re-esterification) and an augmentation of lipid oxidation (Figure 1). These effects may be detected not only by decreases in body fat and in adipocyte size, but also by a decrease in lipid content per adipocyte.[4]

CLINICAL ROLE OF rhGH

Short children are prescribed rhGH to promote linear growth[5] (Table 1) and that is the most visible result of rhGH treatment in infants, children, and adolescents. Additionally, rhGH prevents hypoglycemia in some infants with congenital hypopituitarism. In adults, rhGH is administered[6] to promote physiologic and psychological well-being (Table 2).

Table 2. FDA-approved indications for rhGH therapy in adults

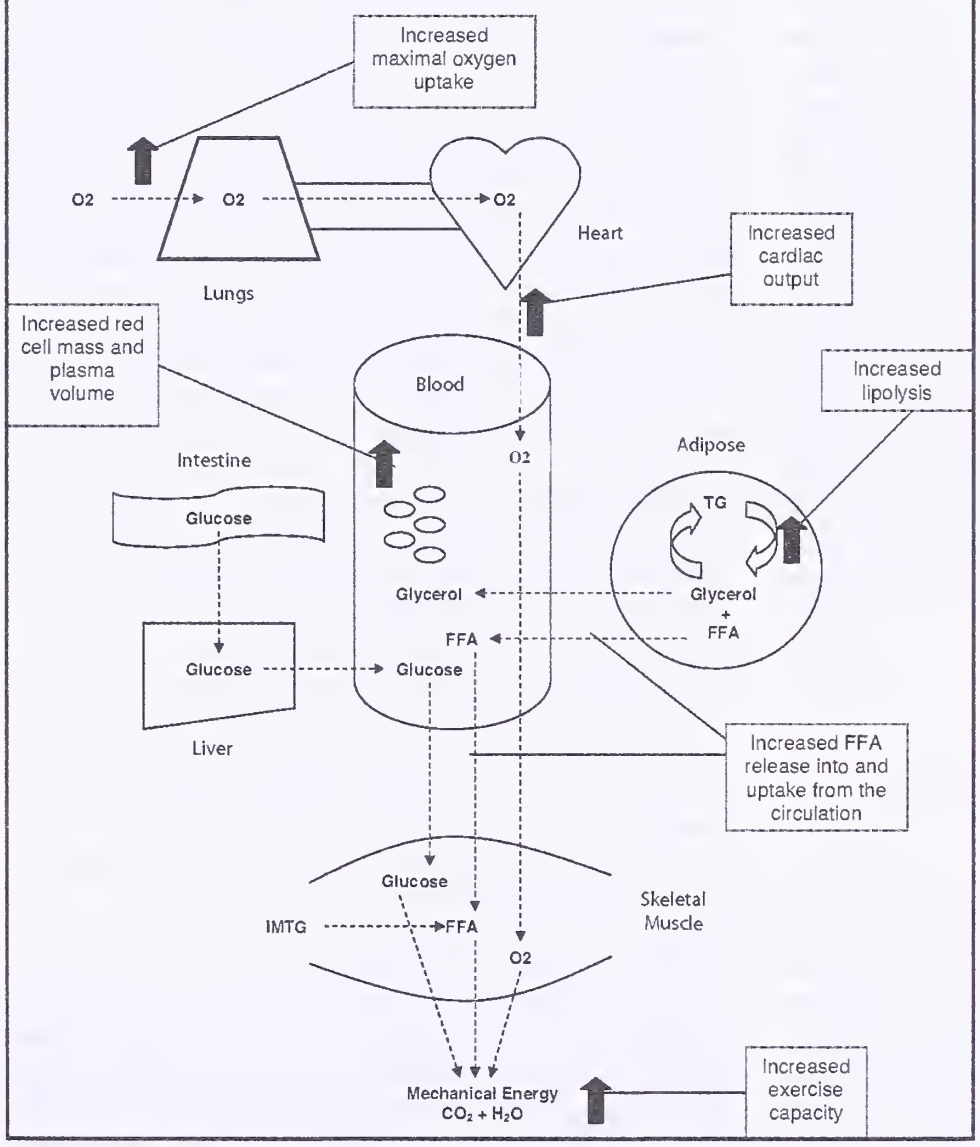
Growth hormone deficiency
Muscle wasting due to HIV/AIDS
Short bowel syndrome

The outcome of rhGH replacement therapy in a GH-deficient child or adolescent may be an increase in fat-free mass, both body cell mass (muscle) and total body water (especially the extra-cellular compartment), and a decrease in body fat with a redistribution from central to peripheral.[7] Controlled experiments in hypopituitary adults have shown that the baseline decrease in functional capacity of approximately 20% reverts to

normal when measured as maximal oxygen uptake (VO_{2max}), aerobic capacity, maximal power output, or ventilation threshold with rhGH treatment.[8,9] The increase in VO_2 was proportionate to the increase in lean body mass (the respiring tissue). A decrease in fatigue was also reported; this is likely due to the decrease in the ventilatory threshold or lactate threshold as a percentage of maximal oxygen uptake. This was perceived as being able to *work* within a comfortable range.

How might this happen? One should note that the requirements for the increase in work require metabolic fuels, oxidation (intermediary metabolism), and useable energy. The immediate burst comes from the oxidation of glucose and the more prolonged capacity from the oxidation of free-fatty acids. By increasing ventilation and oxygen transport by hemoglobin GH directly leads to an enhanced delivery of substrate and oxygen to respiring muscle. Increased cardiac output (stroke volume and left ventricular ejection fraction) permits the distribution of the oxygen to the capillary network and to the extraction of the oxygen by muscle fibers, either to be used directly or stored in myoglobin. Other effects that enhance the delivery of oxygen include diminished systemic vascular resistance. In all of these physiologic responses GH and exercise are likely additive and, perhaps, synergistic. Indirect effects of GH (likely mediated by IGF-I) are related to the alteration in lean body mass and in more efficient thermoregulation.

Figure 1. GH Replacement



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GH AND ATHLETIC PERFORMANCE

In the 1940s the first GH was extracted from human cadaver pituitary glands.[10] The GH that was derived was in such limited quantities that there was none available for the purposes of testing it for athletic performance.[11] Only human and monkey pituitary GH has efficacy in man.[12,13] In 1985 synthesized rhGH received FDA approval. Thus a virtually unlimited supply became available and clinical studies were undertaken in children and adolescents with subnormal growth and in adults with GH deficiency, aging, as well as for performance or aesthetic purposes. The evidence is neither clear nor robust that rhGH produces salutary ergogenic and performance benefits among athletes.[14]

Definition of Doping

The International Olympic Committee (IOC) defines doping as the *"use of an expedient (substance or method) which is potentially harmful to athletes' health and/or capable of enhancing their performance, or the presence in the athletes' body of a prohibited substance or evidence of the use thereof or evidence of the use of a prohibited method"*. There is no mention of intent or of how the substance entered the body. If the substance is in the athlete's body then the athlete is responsible. That is the basis for sanctions for testing positive for a prohibited substance. Sir Arthur Porritt, first chairman of the IOC Medical Commission, noted, *"To define doping is, if not impossible, at best extremely difficult, and yet everyone who takes part in competitive sport or who administers it knows exactly what it means. The definition lies not in words but in integrity of character."*

In fact, there are huge pressures to excel. Athletes are driven to perform their best and along with the pressure to win there is often an attitude that doping is necessary to achieve success. Expectations about success include potentially lucrative financial rewards with winning, such

as collegiate scholarships and salary as a professional athlete. The rationale for taking ergogenic effectors such as rhGH is that by becoming bigger and stronger the athlete will perform better. It should be noted that performance is much more than just strength or endurance; for the athlete must produce, control, and efficiently use the energy in a fashion that maximizes athletic performance.

There is a system in place for therapeutic use exemptions to doping for those athletes who require the *substance* for health; for example, insulin is permissible for those with diabetes mellitus. This is a formal process overseen by the US Anti-Doping Agency (USADA) as the local agent for the world anti-doping effort as directed by the World Anti-Doping Agency (WADA).

Abuse of rhGH

The illegal indications for rhGH are listed in Table 3. Growth hormone is listed under class S2 of hormones and related substances in terms of the 2006 WADA and IOC prohibited list of doping agents. Other peptides in this category include erythropoietin (EPO), corticotrophin (ACTH), IGF-I, and insulin. It is likely that rhGH is being abused at an increasingly prevalent rate. However, it should be noted that much of what is purported to be rhGH—especially products promoted on the Internet—is not. Of course, any drug taken orally cannot be rhGH. Many of the products advertised online and in magazines are GH releasers, mainly amino acids and rarely, analogues of GH releasing hormone (GHRH).[15] It is also worth noting that these are considered "dietary supplements" and not subject to FDA oversight. The notion that amino acids release GH is on solid scientific ground given that tests for GH sufficiency may include arginine, or the closely related amino acid, ornithine. What isn't stated is that very concentrated solutions of these amino acids are administered intravenously before GH is released. Also not prominent is the physiologic concept of the absolute and then relative refractory period following GH release, irrespective of the cause.

Table 3. Off-label/illegal use of rhGH

Anti-aging
Athletic performance enhancement
Body building

A casual Internet search (in June, 2008) using the key words "hGH AND sport performance" yielded approximately 158,000 web links, mainly to sites that had multiple supplements to sell. Many of the listed products require administration for many months. A few examples included:

- Chromium, l-ornithine, l-arginine, l-lysine, l-glutamine, l-glycine, "pituitary" powder, colostrums, placental extract and choline. 60-day supply $49.95
- hGH energizer containing: vitamin B-6, tribulus, l-arginine, l-leucine, l-glutamine, l-lysine, gamma-aminobutyric acid (GABA), l-isoleucine, l-valine, colostrums, l-ornithine and l-glycine. It is touted as an "all natural hGH supplement." 90-day supply $29.95
- A nasal spray. It contains: alpha GPL, GABA, multiple amino acids, many as noted above, l-DOPA, bean extract, momiyo extract and alpha-ketoglutarate—I suspect that many other substances are included! 90 day supply $59.95

Finally, something that might be rhGH for injection, but one must complete a form for a free (medical) consultation and thus presumably for a physician to write a prescription. It is important to note that if a prescription is written for anti-aging, body building, or athletic performance, a felony has been committed by the prescriber, the recipient, and (presumably) the dispenser (Table 3). Cost is not noted, but likely ranges in the $5,000 to $50,000-range depending on the size of the recipient and the dose per kg.

There are many reports that have noted an increasing prevalence of rhGH abuse. These primarily come from anecdotal "information" on the benefits of GH posted on the Internet, as well as a dated, but very favorable write-up in *The Underground Steroid Handbook*,[16] The press has reported an increasing number of seizures from elite athletes including cyclists and swimmers. What is it that athletes expect to obtain from taking rhGH? The athletes want improved performance, but such studies are difficult to do, either as alleged "clinical trials" or observational studies in athletes, for they rarely take agents singularly, but often a "cocktail" of multiple dietary supplements and one or more doping agents.

Although rhGH has not been shown to unequivocally increase muscle strength or to improve performance,[14] it is considered one of the drugs of choice, because it is extremely difficult to prove that one is receiving it. The structure of rhGH is identical to the main isoform of naturally secreted GH. The pulsatile secretion of native GH means that its levels fluctuate widely, from undetectable to clearly within the doping range. Both GH and rhGH have a short half-life in the circulation. Exercise is potent stimulus to GH release and release may be modified by variations in nutrition and legitimate nutritional supplements.

Studies of rhGH in Athletes

Liu and colleagues[14] have systematically reviewed the effects of rhGH on athletic performance. Using stringent criteria for a meta-analysis, they scanned 7599 titles from the largest databases, reviewed 252 abstracts in detail, and retrieved 56 articles for full-text evaluation. Following their review, 44 articles representing only 27

unique studies met the strict inclusion criteria. A total of 303 participants received rhGH for an average of 20 days but a significant number received rhGH only once. The subjects were mainly young men (average age 27 years) and were recreational and not elite athletes. The average dose was 36 µg/kg/day which is approximately 5- to 10-fold the therapeutic dose for adults with GH deficiency. Lean body mass increased in the rhGH-treated groups compared to those not treated (2.1 kg [95% CI, 1.3 to 2.9 kg]) with a small, not statistically significant, decrease in fat mass (–0.9 kg [CI, –1.8 to –0.0 kg]). Body weight did not change significantly. Only 2 studies appropriately evaluated change in strength;[17,18] these were the longest trials of 42 and 84 days duration. On 1-repetition maximum voluntary strength (1-RM) testing, those who received rhGH showed no change in biceps strength (–0.2 kg [CI, –1.5 to 1.1 kg]) or quadriceps strength (–0.1 kg [CI –1.8 to 1.5 kg]). In the second study none of the 7 other muscle groups evaluated showed a positive change in strength.

Minor effects of rhGH have been noted on basal metabolism with a slight decrease in respiratory exchange rate reflecting the preferential burning of fat rather than carbohydrate, at rest. Additionally, very little effect on exercise capacity has been reported. The results may be summarized by noting that lactate levels trended higher, plasma free fatty acid concentrations and glycerol concentrations were significantly increased—reflecting the lipolytic metabolic effect of rhGH—but the respiratory exchange ratio did not change. These studies showed very little ergogenic effects of rhGH in recreational athletes. The studies were of short duration and most likely did not represent how elite athletes administer rhGH, either with reference to dose, duration of doping, or addition of other supplements—both legal and illegal. Based on countless reports in the media, it is clear that many athletes abuse steroids in addition to the *noted* amounts of rhGH. None of the studies would have been able to detect differences of 0.5 to 1.0 % in "performance". These small differences are those that are relevant to the time (track) events, distance or height (field) events that separate the champion from any other finishing position. Similar issues relate to a host of sports other than track and field, but may be even more difficult to quantitate.

Recently, rhGH (19 µg/kg/day) administered for one week was noted to increase strength, peak power output, and IGF-I levels in a group of abstinent dependent users of anabolic androgenic steroids.[19] Great care was taken to be certain that no anabolic steroids were detected in appropriately obtained urine samples. Body weight increased—this was likely water retention—as did peak power output. Although this is a very special group of athletes and is a single study, it was quite carefully performed.

Adverse events were common in the larger group of studies in the Liu et al meta analysis.[14] These mirrored those of adult subjects who administered rhGH in what were at that time, child and adolescent doses. Adverse events included soft tissue edema, joint pain, carpel tunnel syndrome, and excessive sweating. Most were related to fluid retention and considered to be secondary to the rhGH effects on salt and water balance by the kidney.

In a clinical trial designed to determine the pharmacodynamics of rhGH abuse, Nelson and co-workers[20] administered rhGH or placebo and testosterone (in men only) or placebo, or both in a double-blind study to young recreational athletes for 8 weeks. The final doses of rhGH were approximately 4-fold (women) and 6-fold (men) the normally prescribed dose for GH deficient young adults. Although there were no "efficacy" data with reference to body composition or athletic performance, the data are important with reference to adverse events. Although no subject discontinued the study due to adverse events related to rhGH, minor adverse events were reported in all groups, including the placebo groups. Swelling was reported in a greater number of rhGH subjects than placebo subjects (men: 67% versus 2.5%, *P=0.02*; women: 65% versus 31%, *P=0.06*). Subjects receiving rhGH reported more joint pain and *pins and needles* sensations; however, statistical significance was reached only in the men (*P= 0.02* and *P=0.03*). These data show the relatively small "therapeutic" index for rhGH and likely have implications for those athletes purportedly administering much higher doses.

In a clever sub-analysis of the placebo group, only reported in abstract form,[21] this group of investigators queried the placebo group about whether they were receiving active drug. The male athletes who believed that they were administered rhGH, even though they received the placebo, had both *perceived* improvement in performance measures and improvement in one of several *measured* indicators of physical performance. Although the study design[20] was not powered for this endpoint, it certainly does complicate the outcomes of trials with rhGH for performance endpoints.

Virtually all studies reviewed by Liu and colleagues[14] had significant limitations. The major ones included:

- Very few studies evaluated strength and exercise capacity
- Small effects would not have been found
- Short duration of the studies, many for only one dose
- Doses of rhGH and other supplements are very likely different in the real world.

Liu and colleagues concluded, *"Claims regarding the performance-enhancing properties of growth hormone are premature and are not supported by our review of the literature. The limited published data evaluating the*

effects of growth hormone on athletic performance suggest that although growth hormone increases lean body mass in the short term, it does not appear to improve strength and may worsen exercise capacity. In addition, growth hormone in the healthy young is frequently associated with adverse events." [14]

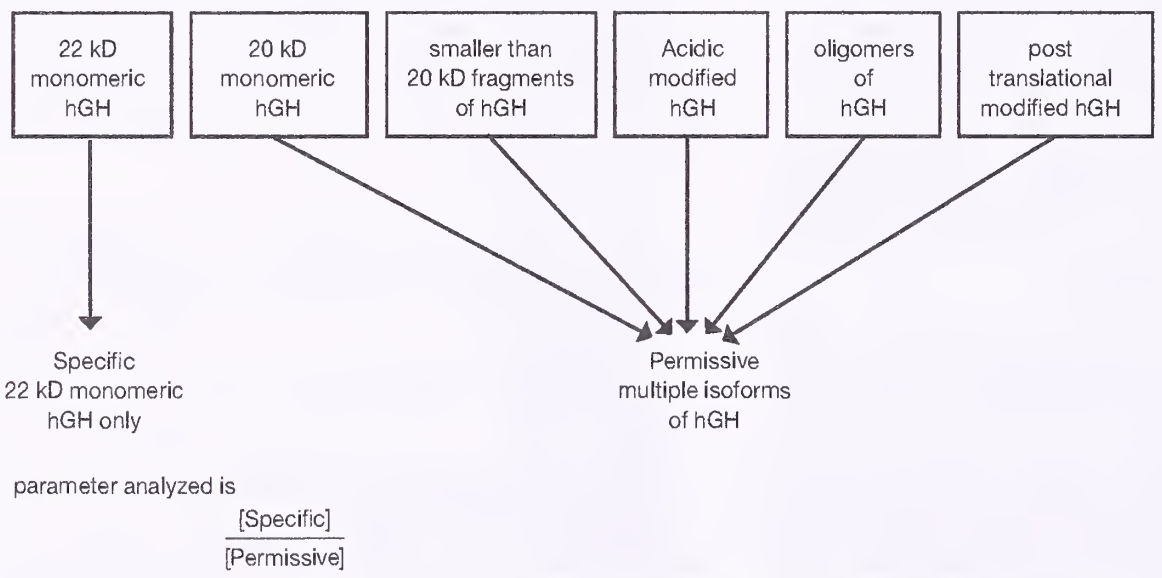
CURRENT DETECTION OF rhGH DOPING

The ability to detect rhGH has been quite a difficult task for analytical chemists, because the amino acid sequence of rhGH is identical to that of the main GH isoform secreted by the pituitary; unlike other peptide hormones it has no N-linked glycosylation sites; its secretion is pulsatile with a short half-life (16 to 20 minutes); there are circulating GH-binding proteins; potential cross reactivity with other peptide hormones (eg, prolactin); and it is stimulated by exercise and stress. Blood sampling is required for all detection methods, because less than 0.1% may be found in the urine. Its renal secretion is poorly understood and greatly variable within and between subjects.[22]

The analytical approaches rely on immunoassays as opposed to the more established doping tests for anabolic steroids, which depend on GC/MS technology (Figure 2). There are 2 general approaches to detection of doping with rhGH. The first, (direct) approach measures the GH isoform composition by the differential immunoassay method.[23] For this approach one constructs pairs of antibodies whose primary focus is all of the isoforms of GH and a second set which is virtually restricted to the 22kD isoform—the one that is 100% of the rhGH. The first assay is called *permissive* (pituitary) and the second *specific* (recombinant). The rationale is that the more one takes of the rhGH (22kD), the less pituitary GH (especially, 20kD) will be secreted; implying that the ratio of the assay of the *specific* to the *permissive* will rise. As an example, the ratio rises from 0.6 to 1.5 in subjects administered rhGH, but this assay would only be valid within a few days of the last injection of rhGH. The validation of this technique requires knowledge (ie, testing) of the effects of exercise on the recombinant/pituitary ratio, an independent confirmatory test, knowledge of the *window of opportunity*, and data from athletes—both recreational and elite. This method is unable to detect doping[22] with pituitary derived GH or the abuse of the GH secretagogues, IGF-I itself or in combination with its major circulating binding protein, IGFBP-3 (IGF-I/IGFBP-3).

The second is the indirect approach in which specific analytes dependent on GH (or IGF-I) are measured. Variables from the IGF system and collagen/bone have been chosen because they change markedly during rhGH administration and it appears that combinations of variables using discriminant functions are the most promising. Detection of rhGH supplementation is possible at least until 2 weeks following the last administration, although there is progressively decreasing sensitivity after the first week. Normative data in athletes have been established.[24] The physiological changes in GH-dependent markers in adolescent athletes are far more dramatic than in older athletes, thus making it quite difficult to detect doping in this age range without constructing a complex algorithm that would depend more on maturational age than it would on the chronological age—another complication for doping control.[25,26] Data using this approach have noted only minor effects due to trauma or micro-injury or ethnic background.[3,27] As with any assay, rigorous standardization is required and interference by concomitant drug abuse, especially anabolic steroids, is a likely complication. For the moment the most informative combination of analytes is IGF-I and procollagen III peptide levels and individual discriminant functions for men and women.

Figure 2. Rationale for detection of hGH in plasma by the differential immunoassay approach



Adapted from Bidlingmaier M, Strasburger CJ. Nat Clin Pract Endocrinol Metab. 2007;3;769-77. Copyright © NPG. 2007. All rights reserved.

FUTURE RESEARCH IN DOPING

The doping-detection field in the future will require the determination of combinations of rhGH-dependent

analytes that remain detectable for a longer period of time than the ones currently available, and perhaps other methods for the direct determination of the IGFs and GH-secretagogues. It would seem that abuse of rhGH (or other peptide hormones) manufactured by the major global pharmaceutical companies could be markedly diminished by adding, for example, an inert fluorescent marker that would be excreted in the urine. Detection of that unnatural marker might then be considered a doping offence. Most likely this would markedly diminish, but not stop, doping offences with these hormones. One can only speculate what is stopping the pharmaceutical manufactures from doing so.

The era of gene doping, for example adding GH or IGF-I genes to specific muscles, is upon us. Experiments have been done in animals.[28] No detection methods presently available could detect this type of doping.

LEGAL ISSUES

As is true for most drugs, physicians may prescribe off-label, meaning that trials for that particular condition have not been performed but that it is logical to use an already approved drug for a specific patient. However, rhGH is quite different; it is illegal to prescribe rhGH off-label for age-related conditions (anti-aging) or for performance enhancement (Table 3). Unlike most FDA-approved medications, rhGH can only be prescribed for indications specifically authorized by the Secretary of HHS (for indications, see Table 1). Because it is not administered orally and it was formerly classified as a drug, rhGH is not considered a dietary supplement and is not subject to the Dietary Supplement and Health Education Act (DSHEA).

The precise language of the Federal Drug and Cosmetic Act[29] (FDCA) under section 303 is:

1. *Except as provided in paragraph (2), whoever knowingly distributes, or possesses with intent to distribute, human growth hormone for any use in humans other than the treatment of a disease or other recognized medical condition, where such use has been authorized by the Secretary of Health and Human Services under section 505 and pursuant to the order of a physician, is guilty of an offense punishable by not more than 5 years in prison, such fines authorized by title 18, or both.*
2. *Whoever commits any offense set forth in paragraph (1) and such offense involves an individual under 18 years of age is punishable by not more than 10 years imprisonment, such fines as are authorized by title 18 or both.*
3. *Any conviction for a violation of paragraphs (1) and (2) of this subsection shall be considered a felony violation of the Controlled Substances Act for the purposes of forfeiture under section 413 of such Act.*
4. *As used in this subsection the term "human growth hormone" means somatrem, somatropin, or an analogue of either of them.*
5. *The Drug Enforcement Administration (DEA) is authorized to investigate offenses punishable by this subsection.*

SUMMARY AND CONCLUSIONS

There are a number of legitimate uses of rhGH in infants, children, adolescents, and adults. It is different from most drugs in that its off-label use is illegal for those unapproved indications related to athletic performance, body building and anti-aging. Although difficult to show any ergogenic advantage in clinical trials, none of the trials have been large enough or have narrow enough end points to have a valid outcome given the changes in performance that are relevant to world-class athletes. Some progress is being made in the ability to detect doping with rhGH, but to date no national or international athlete has been sanctioned for abusing rhGH. This does not mean that rhGH is not being used by athletes, just that the testing is not yet robust enough to capture those abusing rhGH. Further research is clearly needed to improve the detection techniques, and also to determine if rhGH as administered to athletes is actually ergogenic or enhances one's image in body building.

As difficult as it is to note either changes in performance or body composition in adults, it is much more difficult to detect these alterations in adolescent athletes, whose natural pubertal progression involves a marked ramping-up of the GH/IGF-I system, as well as the analytes that are being considered for the detection of doping.

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Editor's Comment:

The lead article in this issue of GGH entitled "Growth Hormone Administration: Is it safe and effective for bodybuilding and improved athletic performance?" by Dr. Alan Rogol reviews the current state of the misguided use of human growth hormone. This topic has been of high interest as prominent professional athletes have been the subject of investigation and hearings by the US Congress. These high profile governmental activities may only be reflecting the tip of the iceberg of a prevalent practice in our society that may be permeating our youth. It is not possible to track the number of individuals receiving illegally distributed growth hormone, but it may account for a $2 billion per year business in the US.[1] This is primarily a cash only business as the vast majority of users pay out of pocket for the drug. The New York State Bureau of Narcotic Enforcement uncovered highly profitable, illegal distribution of growth hormone; an investigated compounding pharmacy purchased 25 grams of imported growth hormone for $75,000 and converted each gram into 3000 IUs of growth hormone, then sold the drug for $6 to $18 per IU—yielding $450,000 to $1,350,000.[2] In 2007, in this case alone, the company entered into a deferred prosecution agreement with the Massachusetts US Attorney's Office and was fined $10.5 million over the illegal distribution of growth hormone for non medical uses. Additionally, there are many other sales through the Internet of multiple products purportedly marketed as growth hormone. These practices preclude the detection and monitoring of adverse events and the potential health consequences of the illegal use of growth hormone.

Pediatric endocrinologists are well acquainted with the wish of children and their parents to administer growth hormone for growth augmentation purposes and are often consulted for its use as an agent for enhancement of their athletic capability and bodybuilding. Beware that the administration of growth hormone for the later purpose is illegal and its efficacy and safety for bodybuilding and athletic performance has not been demonstrated, as discussed in the lead article by Rogol. However, as long as our culture seeks perceived physical enhancements with products like growth hormone, we will have to be aware of the extensive distribution and promotion to our youth and actively participate in curtailing its use. This is quite a challenge, as this and other medications are easily found and sold online. According to The New York Times[3] there are over 365 Internet sites that advertise and/or sell controlled medications by mail and offering to supply the drugs without a proper prescription. The US Drug Enforcement Administration found that 85% of all Internet prescription sales involved controlled drugs, compared with 11% of those filled through traditional pharmacies, suggesting that online

sales are destined for misuse. Mr. Califano, a former secretary of Health and Human Services, said: "Abuse of prescription drugs has exploded among college students, and we think that one way they get these drugs is over the Internet."

Fima Lifshitz, MD
Editor-in-Chief

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REVIEWS & COMMENTS FROM THE LITERATURE

Genetics of Stature

Adult height is primarily (approximately 80% to 90%) determined by hereditary factors. Socioeconomic status, nutrition, and disease influence only a relatively small proportion of attained stature. It has long been suspected that there are a multitude of genes that impact upon this polygenic trait, with each gene exerting an additive but only very limited effect. From genome-wide association studies employing single nucleotide polymorphism (SNP) analyses in approximately 80,000 individuals of European ancestry (UK, Scandinavia, Holland, Iceland), these 3 investigative groups have identified more than 30 chromosomal sites and the potential genes that appear to be partially involved in the regulation of adult stature in humans (Table). Gudbjartsson et al divided the candidate genes into 3 functional groups—those associated with skeletal development (eg, *BMP2, BMP6*), those that encode zinc-dependent metalloproteinases (*ADAMTS10*) and glycoproteins (eg, *FBN1*) that affect cartilage composition, and those that are involved with the processes of chromosome segregation and mitosis (eg, *CDK6*, *HMGA2*). The gene most frequently associated with stature in all 3 studies was *ZBTB38*. This zinc-finger protein binds methylated DNA—specifically the methylated allele of the differentially methylated region of H19/IGF2.[1] This is the site at which epigenetic errors of imprinting result in either the Beckwith-Wiedemann syndrome (OMIM 130650) of somatic overgrowth or the growth retardation syndrome of Russell-Silver (OMIM 180860).[2] ZBTB38 represses transcription of methylated regions. Thus, it is interesting to speculate that ZBTB38 might affect adult stature through regulation of the production of insulin-like growth factor (IGF)-II, perhaps during in utero development when IGF-II is known to be one of the determinants of fetal growth. Independent of its effect on methylated DNA, ZBTB38 also regulates transcription of *TH*, the gene encoding tyrosine hydroxylase, the rate-limiting step in catecholamine synthesis. Other commonly identified gene candidates were *HMGA2* encoding a chromatin architectural factor and *CDK6* encoding a cyclin dependent kinase regulator of the cell cycle.

While each of these candidate genes has only a small effect upon adult height (estimated 0.4 cm), collectively they can exert significant influence and account for only approximately 4% of adult stature. The more "tall" alleles one has, the taller the individual (Figure). In the study of Weedon et al, there was a 5 cm difference in adult stature between subjects with 17 or fewer "tall" alleles compared to those with 27 or more.

Gudbjartsson DF, Walters GB, Thorleifsson G, et al. Many sequence variants affecting diversity of adult human height. Nat Genet. 2008;40:609-15.

Lettre G, Jackson AU, Gieger C, et al. Identification of 10 loci associated with height highlights new biological pathways in human growth. Nat Genet. 2008;40:489-90.

Weedon MN, Lango H, Lindgren CM, et al. Genome-wide association analysis identifies 20 loci that influence adult height. Nat Genet. 2008;40:573-83.

First Editor's Comment: *These reports are of great interest as they dramatically illustrate just how many genes must be involved in the determination of adult stature. They also illustrate the quantitative problem that the clinician will face in identifying the "cause" of genetic short stature in a specific patient. However, it was difficult to critically examine the data because some of it was derived by meta-analysis of previously published reports. Thus, it was unclear whether or not there may have been some overlap between analytical data utilized in the 3 reports. The reports are also difficult to interpret because the investigators employed different probes for similar or related SNP sites. For example, ZBTB38 was identified as SNP rs724016 in the report of Lettre et al, as SNP rs6440003 in the report of Weedon et al, and as SNP rs6763931 in the report of Gudbjartsson et al. [A brief expository review of genome-wide association studies and SNPs has been written by Christensen and Murray.[3]]*

Allen W. Root, MD

Second Editor's Comment: *Fisher proposed in 1918 that many genetic factors, each having an individually*

Chromosome loci and candidate genes highly associated with adult stature

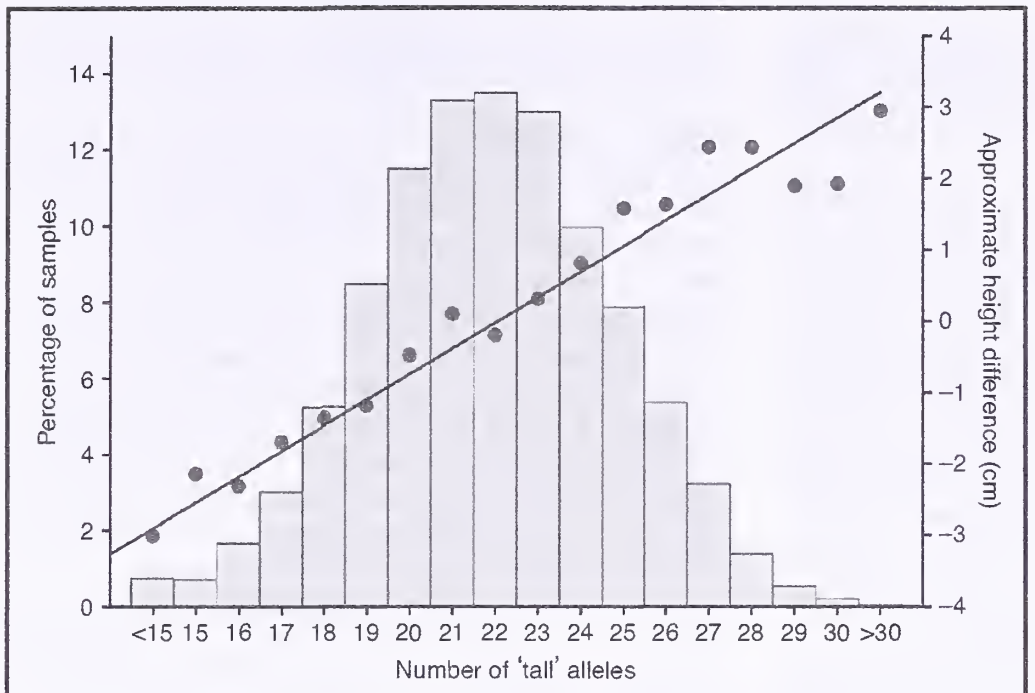
Chromosome	Gene	Mutated Human Disease (OMIM)	Function
2	*EFEMP1*	601548	Fibrin-like matrix protein. Retinal dystrophy (126600)
3	*ZBTB38*	-	Binds to and represses methylated DNA
4	*LCORL*	611799	Transcription Activator
4	*HHIP*	-	Regulates hedgehog signaling
6	*LIN28B*	606178	Promotes cell growth
6	*BMP6*	611044	Bone morphogenetic protein
6	*GPR126*	112266	Orphan G protein receptor
7	*CDK6*	603368	Cyclin dependent kinase-regulator of cell cycle
7	*GNA12*	604394	Guanine nucleotide binding protein-with mitogenic properties
9	*PTCHI*	601309	Receptor for Sonic, Indian & Holoprosencephaly (610828) Desert hedgehogs. Basal cell nevus syndrome (109400)
12	*HMGA2*	600698	Chromatin architectural factor. Tall stature, lipomas
12	*SOCS2*	605117	Suppresses cytokine signaling – via Janus kinase and signal transducer and activation of transcription (STAT)
14	*TRIP11*	604505	Interacts with TRβ/T3
15	*ADAMTSL3*	609199	Component of extracellular matrix
15	*AGC1**	155760	Aggrecan – chondroitin sulfate. Spondyloepiphyseal dysplasia – Kimberly (608361) proteoglycan core protein
15	*FBNI*	134797	Fibrillin-connective tissue matrix. Marfin syndrome (154700)
18	*DYM*	607461	Transmembrane protein Osteochondrodysplasia (607326, 223800)
19	*DOT1L*	607375	Histone-3 methyltransferase
19	*ADAMTS10*	60899	Metalloproteinase. Weill-Marchesani syndrome (277600)
20	*GDF5*	601146	Cartilage morphogenetic protein, Chondrodysplasia (201250, 200700, 113100), TGFβ subfamily
20	*BMP2*	112261	Bone morphogenetic protein, stimulates bone formation

*Designated *ACAN* in reports
(Data culled from the reports of Lettre et al, Weedon et al, and Gudbjartsson et al.)

small effect, explain the heritability of height.[4] Much attention has been devoted since that time to identifying these factors. For instance, numerous genes have been identified that harbor mutations responsible for the osteochondrodysplasias and other syndromes associated with severe short stature, but in general these genes do not seem to influence the normal continuous variation in stature. Although linkage studies have elucidated chromosomal regions that affect height variation, they have not identified specific gene loci that influence height in the general population. It has not been until the recent application of genome-wide association (GWA) studies that significant headway has been made. This approach takes advantage of high-throughput analysis of single nucleotide polymorphisms (SNPs) identified through the so called HapMap project, a growing number of patient groups for whom DNA is available for analysis and advances in computational methods that enable such analysis and permit datasets to be combined. Indeed, one of the first GWA investigations of height was reviewed in GGH.[5] This reviewed study has now been expanded substantially and joined by 3 other large GWA studies as reported in the May 2008 Nature Genetics. The new investigations have utilized more rigorous multi-stage experimental designs to analyze hundreds of thousands of SNP markers in ~63,000 individuals measured for adult height.

The report by Weedon et al identified 20 genetic variants which, in the aggregate, account for ~3% of height variation in adults of European ancestry. The identified SNP markers do not influence height per se, but they implicate genes within which or nearby to which they reside. One can envision how most of the candidate genes implicated in this manner could influence growth as they encompass growth factors and their receptors, proteins that interact with or alter the extracellular milieu of growth factors and proteins that modulate intracellular signaling or are linked to cell cycle regulation or cancer. Most notable here are Indian hedgehog (IHH), Hedgehog interacting protein (HHIP) and Patched 1 (PTCH1), which belong to the Hedgehog pathway, growth and differentiation factor 5 (GDF5), suppressor of cytokine signaling 2 (SOCS2) and cyclin-dependent kinase-6 (CDK6). The previous association with a marker near the high mobility group-A2 (HMGA2) gene locus was confirmed.

The report by Lettre et al identified 10 loci associated with height variation also in adults of European ancestry, 4 of which were the same as in the Weedon report including HHIP. These authors emphasized that 3 of the candidate genes—HMGA2, the histone methyltransferase DOT1L and the methyl-DNA-binding transcriptional repressor gene ZBT38—are involved in chromatin remodeling. They



The combined impact of the 20 SNPs with a $P < 5 \times 10^{-7}$. Subjects were classified according to the number of 'tall' alleles at each of the 20 SNPs; the mean height for each group is plotted (blue dots). The black line is a linear regression line through these points. The gray bars represent the proportion of the sample with increasing numbers of 'tall' alleles. The approximate height difference (cm) was obtained by multiplying the mean Z-score height for each group by 6.82 cm (the approximate average s.d. of height across the samples used in this study).

note that the 3' untranslated region of HMGA2 contains the largest number of let-7 microRNA binding sites and that 3 of the other implicated genes, CDK6, DOT1L and LIN28B, a gene upregulated in hepatocellular carcinoma, are considered targets of let-7. MicroRNAs, such as let-7, are small, nontranslated RNAs that down regulate expression of target genes.

The report by Gudbjartsson et al detected 27 genomic regions in which SNP variants were associated with adult height. Their data came from individuals with Icelandic, Dutch, European- and African-American ancestries and results accounted for 3.7% variation in adult height. Several of the implicated genes were the same as in the other 2 reports, but a few additional genes were indentified including BMP2, BMP6 and the TGF-ß and BMP inhibitor, Noggin (NOG).

In contrast to the GGH abstract[5] describing a single SNP association with adult height published in May 2008, these new reports identify 54 gene loci that influence variation in height in adults primarily of European descent. As noted in the accompanying editorial by Visscher,[6] it is reassuring that SNPs previously observed to associate with height were confirmed, SNPs in 3 genes were found associated with height in all 3 studies, and 7 genes were implicated in 2 of the 3 investigations. It is not surprising that variation in genes involving growth factors or modulation of growth factor signaling pathways influence height. More intriguing and novel is the implication of genes involved in chromatin remodeling and in microRNA regulation of gene expression. The papers illustrated the power of GWA studies and also the necessity of very large sample sizes creating consortia of research groups and even consortia of consortia as stated by Visscher.[6]

William A. Horton, MD

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Gender of Growth Hormone Recipients in the US and Globally

The investigators examined gender-based patterns of recombinant human growth hormone (rhGH) use in the US and how it compares to that of other countries, in the context of findings of previously reported gender disparities and the fact that rhGH has entered its third decade of clinical use. Data from all patients enrolled in the International Growth Study (KIGS) registry were included in the analysis. Patients were categorized into 4 geopolitical regions: US; Europe/Australia/New Zealand; Asia; and Rest of the World (ROW; Argentina, Brazil, Colombia, Egypt, El Salvador, Guatemala, México, South Africa, and Venezuela). The US portion of the database was further divided into 10 geographic regions, according to US Postal Service zip code. To minimize the diagnostic inconsistencies across investigators, geographic regions and time, over 100 KIGS diagnoses were collapsed into 8 categories: (1) congenital GH deficiency; (2) organic acquired GH deficiency; (3) renal insufficiency; (4) Turner syndrome; (5) Prader Willi syndrome (PWS); (6) small for

gestational age (SGA), intrauterine growth retardation (IUGR); (7) familial short stature/constitutional growth delay/idiopathic short stature (FSS/CGD/ISS); and (8) idiopathic, neurosecretory, and transient GH deficiencies (IGHD).

Analyses depicted a consistent male predominance among US pediatric rhGH recipients, at almost 2:1. The gender ratio did not change significantly across the 3 time periods defined by the sequence of FDA-approved indications: 1992 and before comprised the classic GH-deficiency era; 1993-2000 the non-GH deficiency pathophysiology era; and 2001 onwards, the height-based era. All indications except PWS (and Turner syndrome because it is female-limited) significantly exceeded 50% males. The male predominance for all non-organic indications combined (72%) exceeded that for the organic indications, with or without Turner syndrome (38% and 59% male, respectively; *P<0.0001* for both). Males outnumbered females at all ages, but with increased disparity during the second decade. With regard to male predominance across US regions, the areas with maximal and minimal percentages differed for each indication and the predominance did not correlate with either the number of children in each geographic area or the ratio of pediatric endocrinologists to children in each area. Comparing the US with global patterns demonstrated the US to have the second greatest male predominance, exceeded by Asia (mostly Japan), but greater than Europe/Australia/New Zealand. Recipients of rhGH in the ROW region were only 47% male.

The authors concluded that male predominance among US pediatric rhGH recipients, described at the introduction of rhGH, persisted into this third decade of use. The factor that most consistently affected the gender distribution was the diagnostic indication, with the greatest disparity appearing in indications without clear organic etiologies, ie, ISS. The absence of male predominance in the ROW region raises the question of cultural influences on rhGH use. The authors also noted that of the 10 greatest rhGH users, the US is the only country with a commercial third-party payer health system as well as being the only country in which ISS became a government-approved indication for pediatric rhGH therapy. The investigators concluded that medical care providers need to be aware of the reported practice bias, and carefully consider girls with growth failure to ensure timely diagnosis and treatment of underlying health problems.

Grimberg A, Stewart E, Wajnrajch MP. Gender of pediatric recombinant human growth hormone recipients in the United States and globally. J Clin Endocrinol Metab. 2008;93:2050-6.

Editor's Comment: *The persistent trend in the disproportionate number of males treated with rhGH should raise a number of concerns as well as provoke questions regarding the likely cosmetic (rather than medical necessity) rationale for rhGH treatment. Grimberg and colleagues previously reported that girls were referred for short stature half as often as boys and were more likely to have an identifiable underlying condition.[1] It could be concluded that in the shift from monitoring growth, as a general indicator of physical health to measuring height and treating short stature, that girls, in general, are placed at higher risk of having serious medical conditions diagnosed later than boys.*

Given the incremental cost-effectiveness of rhGH therapy for ISS is approximately $52,000 per inch,[2] it is well worth pondering why the US is the only country in which ISS became a government-approved indication for pediatric rhGH therapy. As Grimberg and colleagues noted, the data from this study suggest that social and cultural differences, in conjunction with perceived acceptability of rhGH expenditures, foster greater gender disparities in pediatric rhGH use in Japan and the US compared with other world regions.

One of the largest gender disparities was found to be within the category of rhGH initiation starting at 15-20 years of age. Multiple studies have demonstrated that age of rhGH initiation is one of the best predictors of growth response, with the younger the age at initiation, the better the response.[3,4] Initiating rhGH treatment in boys significantly more than girls between the ages of 15-20 years, with the knowledge that replicated clinical findings predict minimal growth response outcomes for this age range, lends support to the interpretation of over-treatment of boys.

David E. Sandberg, PhD

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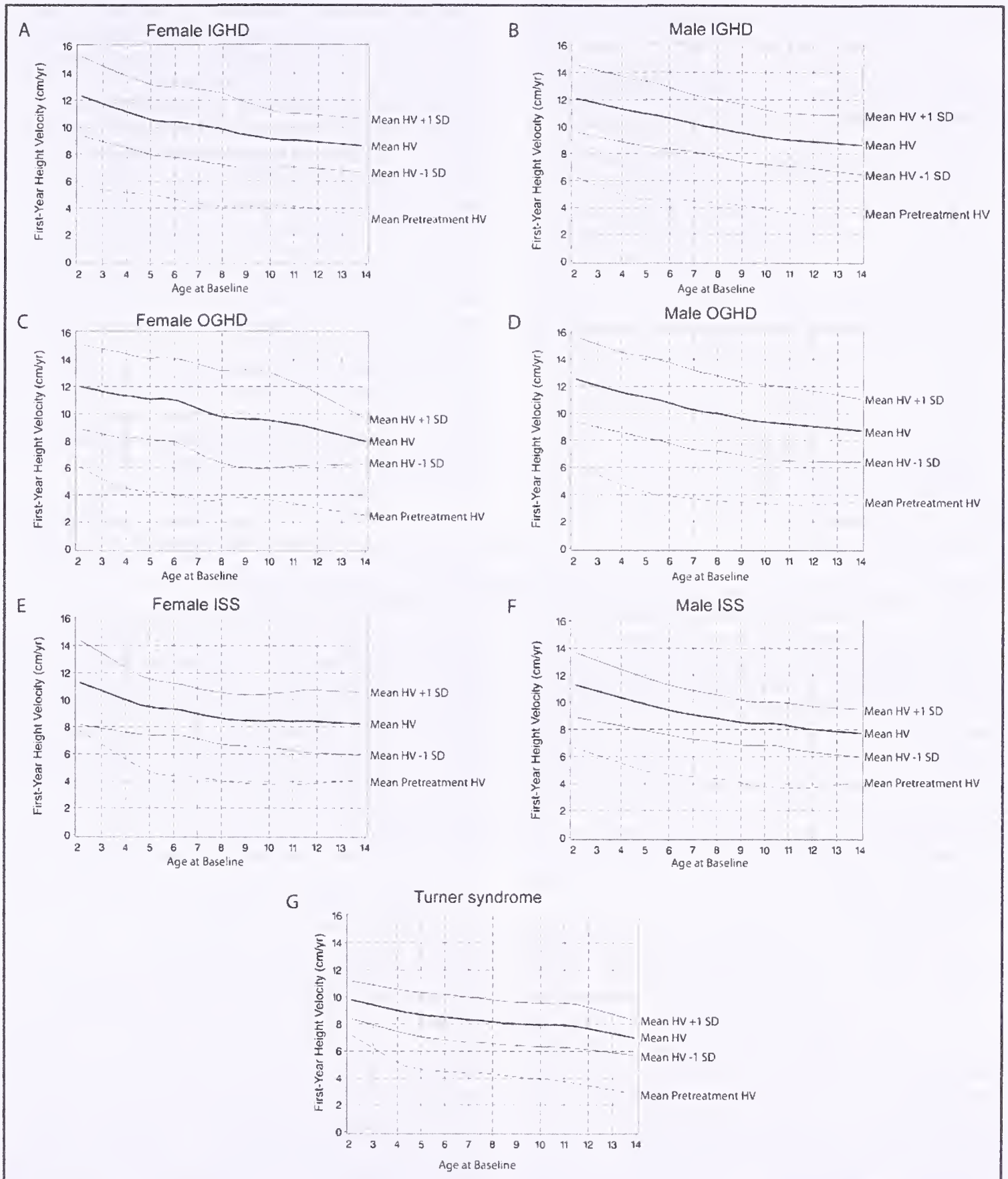
Height Velocity Targets for First Year Growth Hormone Responses in Short Children

Recombinant human growth hormone (rhGH) has been used for treating a number of conditions. Several attempts have been made to define and predict GH response with the development of mathematical models, mostly in GH-deficient (GHD) patients. Such models do not account for the variability observed in GH responsiveness (or sensitivity) in different types of short stature. Therefore, the authors presented evidence-based data criteria for defining the GH responsiveness. Their aim was to provide clinicians

with age-specific targets, considering the first year of treatment with standard daily doses of rhGH in prepubertal short children. Using data from the National Cooperative Growth Study (NCGS), GH response curves were constructed for the first year of treatment. All children were new to treatment and prepubertal. Data were collected from 4297 boys and 3061 girls with idiopathic/organic GHD, idiopathic short stature,



First-year growth responses to daily GH expressed as HV at age of treatment onset (x-axis) in naive, prepubertal females and males with IGHD (A, B), OGHD (C, D), and ISS (E, F), and females with TS (G). Data given for mean and mean ± 1 SD.

and Turner syndrome. All data were cross-sectional, mean +1 SD for first year height velocity (HV) on rhGH were plotted (in cm) against subject age at onset of rhGH treatment, as well as the mean pre-treatment HV data. Height velocity plots of each category as a factor of age at baseline were developed. Mean –2 SD HV plots approximated the pre-treatment HV. The results were presented in a series of plots; these were primarily graphical. Interestingly, each graph contained the curve for the treatment growth velocity of different stages and types of patients (Figure).

There was considerable variability in the response to therapy with rhGH in children receiving a standard weight-based dosing schedule. The wide range of clinical responsiveness to therapy may also denote a challenge to the traditional fixed weight-based dosing generally employed. It emphasized the importance of age at initiation of treatment. Another point of interest was the similarity of the growth response pattern across etiologies of short stature. These growth response curves should also be viewed as conservative and to include some limitations, ie, an unknown amount of non-compliance in all groups, GHD may be part of multiple pituitary deficiencies, etc. Nevertheless, it is suggested that these data offer the clinician a tool to assess progress of an individual patient within an evidence-based frame.

Bakker B, Frane J, Anhalt H, Lippe B, Rosenfeld RG. Height velocity targets from the National Cooperative Growth Study for first-year growth hormone responses in short children. J Clin Endocrinol Metab. 2008;93:352-7.

Editor's Comment: *These data, derived from a large population study, are welcome and provide a context of the well known large variability in growth responses to rhGH therapy. The growth response graphs provided an additional but useful tool in contrast to numerous previous studies which proposed more theoretical and mathematical predictive approaches: prediction of the first year response to rhGH and prediction of adult height based on the first year growth response. These mathematical models were also derived from post-marketing long-term follow-up data, but they did not provide us with practical tools in clinical practice. The new growth response curves showing the first year of therapy may help evaluate the initial catch-up growth and the adjustment of rhGH doses after the first year of therapy. This could be performed in relation to age. In any case, they focus our attention on the first year of treatment and provide information which may turn out to be useful for the patient and the family, particularly in the group of idiopathic short stature patients and in those that may have compliance problems.*

Raphaël Rappaport, MD

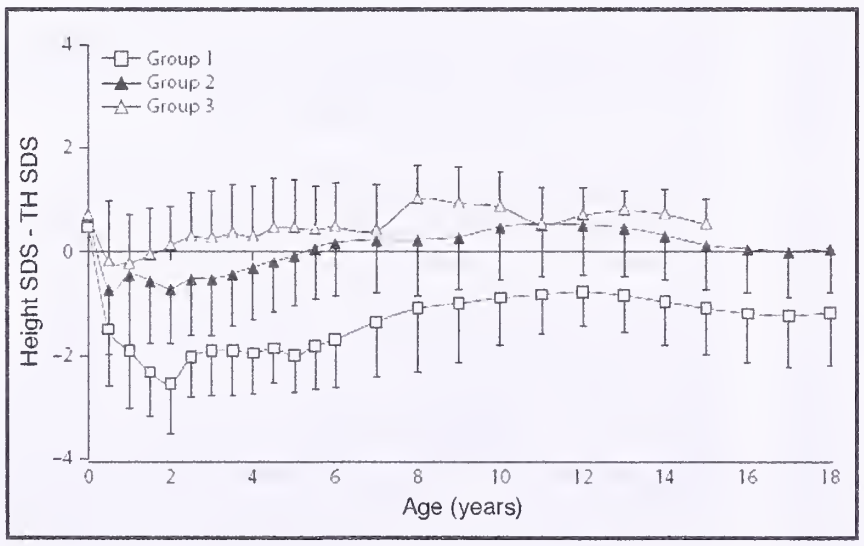
Adult Height of Treated Congenital Adrenal Hyperplasia Patients

Noting reports indicating that patients with the 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) often fail to reach their target height, Hoepffner and colleagues reviewed medical charts of 56 patients with 21-hydroxylase deficiency to examine the effects on final height of strictly controlling hydrocortisone and fludrocortisone. Sixty-two patients were followed continuously at a university children's hospital by the same physician; 6 were excluded from analyses due to receiving additional medications that may effect CAH therapy and/or growth (n=2) or due to bilateral adrenalectomy (n=4).

Participants were divided into 5 subgroups. Patients in the first 3 groups were diagnosed within their first year of life: (1) adult patients born before 1975 (n=13, all salt-wasting, 5 males); (2) adult patients born in or after 1975 (n=26, 21 salt-wasting, 8 males); (3) 7 to 15 years of age and had not yet attained final height (n=9, all salt-wasting, 4 males); (4.1) pre-pubertal bone age, late diagnosed, therapy initiated at 3.5 to 6 years of age (n=5, 3 salt-wasting, 3 males); and (4.2) pubertal bone age, late diagnosed, therapy initiated at 5.5 to 9 years of age (n=3, 1 salt-wasting, 2 males). All patients received therapy monitoring exclusively by the outpatient unit and had regular 3-month measurements of height, weight, blood pressure, and bone age (BA). Hydrocortisone was administered 3x/day, every 8-10 hours. Management was designed so the course of BA followed the course of chronological age (CA), ie, patients received an increased hydrocortisone dose if BA was higher than expected over a 6-month observation period, and vice versa. Fludrocortisone was administered 2-3x/day with the hydrocortisone. Dosage was monitored by blood pressure; to avoid fludrocortisone overdosage, blood pressure values were not allowed to exceed the upper normal range. Change from DOCA to fludrocortisone was gradually introduced to patients in groups 1, 4.1, and 4.2 since the late 1970s when the current therapy regime was initiated. Authors provided specific hydrocortisone and fludrocortisone dosages by age group within group. Beginning in 1992, morning 17-hydroxyprogesterone (17-OHP) in saliva was measured by immunoassay every 3 months and, sometimes more frequently after reaching 5 to 6 years of age. Occasional plasma rennin concentration measurements were added to the monitoring regime. Target height standard deviation scores (htSDS) were based on measured parental height. All values for data analysis were collected via retrospective chart review. Statistical analyses focused mainly on groups 1 and 2. The authors recommended that readers consider results pertaining to groups 4.1

and 4.2 as clinical observations due to the small sample size and to view group 3 results as a demonstration of current corticoid dosage since patients had not reached their final height.

Results showed patients in group 1 had a mean corrected height (ie, measured htSDS minus target htSDS) of approximately –2SDS during their first years of life; this increased to approximately –1 htSDS by age 8 where it remained through adolescence to adulthood (ie, 18-years old) (Figure). In the 1st, 2nd, 4th, and 5th years of life, group 1 growth rates were significantly less than in group 2; in each year (0 to 18 years), the mean group 1 htSDS was significantly lower than that for group 2. With regard to bone age, group 1 experienced significant suppression between ages 1 to 6 years compared with the BA SDS in group 2, followed by a recovery to 1.8 SDS at age 8. Regarding BMI, patients in group 2 showed a continuous and statistically significant increase from ages 2 to 8 years to approximately 1 SD, with no increase after this age. Patients in group 2 reached their target height (0.1 corrected final htSDS). The authors noted that the corrected final mean htSDS of –1.2 of group 1 (those born before 1975) was similar to values reported in the literature and was due to likely excessive corticoid dosages particularly during the first 2 years of life. Use of lower corticoid doses during the following years of life resulted in extremely fast bone maturation up to approximately 1.8 SD, which exceeded growth velocity. In contrast, combined early treatment involving substitution therapy with hydrocortisone and fludrocortisone (ie, group 2) was associated with patients reaching their target height. A similar pattern emerged in groups 3, 4.1, and 4.2. Hoepffner and colleagues credit success with attaining target height to strict medication adherence and monitoring, specifically, by keeping BA the same as CA through combined corticoid administration every 8 hours. For patients with classic CAH who are treated early and following the recommended regimen, they see no need for other forms of therapy (eg, growth hormone (GH), gonadotropin-releasing horomone analogs [GnRHa], antiandrogens, or aromatase blockers).



Pattern of corrected height SDS (height SDS – target height SDS) ± SD of patients of groups 1–3. Reprinted with permission from Hoepffner W, et al. Horm Res. 2008;70:42-50. Copyright © Krager 2008. All rights reserved.

Hoepffner W, Kaufhold A, Willgerodt H, Keller E. Patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency can achieve their target height: The Leipzig experience. Horm Res 2008;70:42-50.

First Editor's Comment: *At a time when different medications are introduced in an effort to assist children with CAH attain their target heights,[1,2] Hoepffner and colleagues findings are quite exciting. Children with CAH can attain their target height with strict monitoring and careful hydrocortisone and fludrocortisone administration. This finding is particularly important as we discover more about the risks of various alternative strategies to increase height. While a full review of all medication side effects is not possible here, I offer the following for thought: GnRHa treatment, in conjunction with GH, is one tool used to arrest pubertal progression in persons with CAH and, thereby, prolong the time over which linear growth can occur. The use of this medication is, however, not without drawbacks. In a study of visuospatial working memory pre- and post-GnRHa treatment in young women, results suggested that hormone withdrawal following GnRHa administration alters the neural circuitry underlying performance of the visual working memory.[3] Specifically, although behavioral responses appeared unimpaired, event-related fMRI under GnRHa exposure was found to be associated with attenuated left precuneus and posterior cingulate cortex activation at encoding and cerebellar activation at recognition. These effects were observed at an 8-week assessment. It could be argued that these changes may return to pre-GnRHa-treatment levels following discontinuation; however, alterations to typical brain function during adolescence (the developmental stage at which GnRHa would be administered to youth with CAH) may have organizational effects that extend beyond the point GnRHa is withdrawn. Evidence for this possibility comes from experimental research by Schulz and Sisk[4] which demonstrated that pubertal gonadal hormones act in lasting ways on the juvenile brain. For example, the authors showed that male Syrian hamsters deprived of gonadal steroid hormones during their pubertal phase of development failed to demonstrate typical masculine reproductive behavior even when those hormones were later replaced. They found that adolescent exposure to testicular hormones causes male behaviors that communicate moment-to-moment dominance status between animals. Similarly, adolescent exposure to ovarian hormones defeminizes female reproductive behavior in the Syrian hamster. Comparable "organizational" effects of steroid hormone*

exposure during adolescence are seen in female rats. Species differences notwithstanding, there is as yet no basis to guarantee families of the long-term safety associated with experimental protocols such as GnRHa, antiandrogens, or aromatase blockers to optimize height. It is therefore reassuring to learn that patients with CAH can achieve their target height through vigilant surveillance of hormone replacement alone.

David E. Sandberg, PhD

Second Editor's Comment: *The paper by Hoepffener et al clearly illustrates that CAH patients who receive an appropriate treatment usually attain their target height, whereas those who do not receive the best medications or do not follow a strict adherence and monitoring of the treatment regimen may not reach their genetically determined height. This observation is very important and should raise our awareness of the difficulties that patients face with demanding long-term treatment protocols for chronic conditions like CAH. When such a patient's growth is faltering additional therapies ie, GnRHa may compound the problem, not withstanding cost, and may lead to other potential concerns. Dr. Sandberg's commentary focuses on new experimental data that suggested that manipulation of the timing of puberty can affect neuroendocrine function and behavior, at least in animals. These potential effects need be investigated in patients with precocious puberty who are regularly treated with GnRHa. In patients with CAH, as well as in others with chronic conditions, the first challenge is to deal with the adherence and compliance of the patients. This issue was reviewed in GGH[5] and despite the importance of medication in treatment, compliance ranged from 11% to 93%.[6] Lack of response or inappropriate response to medication may be indicators of poor adherence.[7]*

Fima Lifshitz, MD

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Combined GH and Aromatase Inhibitor Therapy in GHD Adolescents

In this study, Mauras et al investigated whether the potent aromatase inhibitor, anastrozole, could delay bone age acceleration and increase adult predicted height in boys with growth hormone deficiency (GHD). They studied 52 GHD adolescents on recombinant human GH therapy who were randomized to co-treatment with anastrozole or placebo daily for 36 months. Fifty subjects completed 12 months, 41 completed 24 months, and 28 completed 36 months of treatment. Bone age advancement was significantly slower in the anastrozole vs the placebo group both at 2 and 3 years of therapy (1.8 ± 0.1 vs 2.7 ± 0.1 yr and 2.5 ± 0.2 vs 4.1 ± 0.1 yr, respectively [$p<0.0001$]). This resulted in an increase in predicted adult height of +4.5 ± 1.2 cm at 24 months and of +6.7 ± 1.4 at 36 months of therapy in the anastrozole group when compared with a 1 cm gain at both time points in the placebo group. While serum testosterone concentrations increased more in the anastrozole group after 12 months of therapy, this difference was not significant at 24 months. Estradiol and estrone concentrations increased gradually in the placebo group during the 3 years of treatment, while they remained stable in the anastrozole treated group. Insulin-like growth factor (IGF)-I levels were similar at baseline and increased in a similar fashion in both groups throughout the study. The pace of pubertal progression was similar between groups as measured both by testosterone concentrations and testicular volumes. Fasting lipids, glucose concentrations, complete blood count, urinalysis and liver profiles were normal in both groups at baseline, with no significant differences over time. There was no difference in lumbar spine bone mineral density between groups. The authors concluded that anastrozole increased the adult height potential of adolescent boys on GH therapy while maintaining a normal pubertal progression.

Mauras N, Gonzalez de Pijem L, Hsiang HY, et al. Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years. J Clin Endocrinol Metab. 2008;93:823-31.

Editor's Comment: *Treatment with GH has been shown to improve the final height of GHD children. However, once puberty has begun the time available to increase linear growth in GHD patients is limited. Gonadotropin releasing hormone (GnRH) analog therapy in addition to GH treatment has proven to improve the near final height of GHD patients in puberty.[1] However, this form of therapy is relatively expensive, requires long-term parenteral administration and careful follow-up, and recent studies have demonstrated substantial changes in body composition and in intermediary metabolism, with an increase in adiposity and a decrease in protein synthesis, lipid oxidation, energy expenditure and muscle*

strength following analog use.[2] Additionally, prior studies by Mauras et al[3] have demonstrated increased loss of urinary calcium and in the rate of calcium resorption, with a significant decrease in measures of bone formation in treated patients.

In this study, Mauras et al demonstrated how combined anastrozole (a potent aromatase inhibitor which blocks the conversion of androstenedione to estrone and testosterone to estradiol) and GH administration to adolescent GHD boys increased their adult height potential. Growth velocity remained similar to that of GH and placebo treated children, but with a slower increase in bone maturation, resulting in a net increase in predicted adult height. Anastrozole was well tolerated and free of side effects with no negative effects on fasting lipids or glucose, liver function tests or changes in fat-free mass or percent fat mass. While the increase in lumbar bone mineral density was less in the anastrozole treated group at 24 months, this difference was not noted at 36 months and osteocalcin concentrations, a measure of bone formation, were similar during the whole treatment period in both anastrozole and placebo treated patients. The pace of pubertal progression as determined by changes in testosterone concentrations and in testicular volumes was also similar in both groups. Therefore, treatment with an aromatase inhibitor and GH may offer an alternative in promoting growth in GHD boys who have entered into puberty. However, this conclusion is based on limited data, as patients have not been followed to final height, studies have been performed only in males and in a limited number of patients, and only one randomized double-blind, placebo-controlled study has been performed in this group of patients. More long-term data regarding bone health, potential effects on spermatogenesis and sperm motility, lipid and carbohydrate metabolism will be necessary in assessing the pros and cons of aromatase inhibitor therapy in GHD children and in short boys in general.

Roberto Lanes, MD

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Factors Predicting Ante- and Postnatal Growth

In an attempt to better understand factors which contribute to both antenatal and postnatal growth, a series of conditional analyses were performed on data collected prospectively from 1218 mother and infant pairs. The study subjects had to be Caucasian, have a prenatal visit before 20 weeks, and had to demonstrate a structurally normal single fetus which was carried to term. Maternal steroid use or thrombotic disorders disqualified the pairs. At the first prenatal visit maternal height was measured as well as paternal height when available. Maternal weight was measured and the history was taken with regard to tobacco use. Socioeconomic status was determined from information regarding education, marital status, occupation, partner's occupation, and social class. Assignment was made using the classification system of the UK Office of Population, Census, and Surveys. Placental weight was recorded after the membranes were trimmed. Birth weight was measured using self-calibrating scales, length by infantometer, and head circumference with a metal tape. In addition, skin fold measurements were made at the triceps, subscapular, and quadriceps areas. These measurements were repeated at 6 months of infant age. Feeding practices were noted at birth and reassessed at 3 months. These were classified as totally breast fed, mixed, or totally bottle fed.

The cohort of women in this study was not different anthropometrically from the UK population and social class distribution was also representative. Non-smokers comprised 71% of the cohort. Placental weight was shown to be related to birth weight, birth length, and head circumference. Factors determining placental weight, when birth weight was excluded, were gestational age at delivery, maternal height, weight at first prenatal visit, and paternal height. One factor—increasing parity—had a negative effect. These factors explained 7% of the variance in placental weight. When the analysis was redone including birth weight, length of gestation, and smoking during pregnancy, these also influenced placental weight in a positive manner. Female gender was associated with reduced placental weight. These factors explained 40% of the variance in placental weight.

Placental weight, parity, maternal weight at first prenatal visit, and gender of the infant did not influence weight, length, or head circumference at 6 months of age. Weight at 6 months was influenced by maternal and to a lesser extent paternal height. Smoking was associated with a relatively heavier infant at 6 months and lower socioeconomic status had an additional effect as did breast-feeding. Duration of pregnancy was also an important factor. Length SDS at 6 months was influenced by maternal and paternal height. Smoking had no effect on length at 6 months. Head circumference at 6 months was influenced by maternal weight, height, duration or pregnancy, and maternal smoking whereas breast-feeding at 6 months was associated with a reduction in

head circumference SDS.

These data demonstrate an important impact of maternal and paternal stature on the size of the infant at 6 months. Parity, placental weight, and birth weight, although important to the size of the infant at birth, have little effect on growth during the first 6 months. The effect of parity is mediated by determination of size of the infant at birth and this is mostly mediated by placental weight. The findings demonstrated that small and large babies have small and large placentas respectively. The authors pointed out that the factors that might be modified to determine placental weight and therefore size at birth are rather limited, the most important being smoking. Smoking during pregnancy is associated with a lower birth weight, shorter length and reduced head circumference. But there is compensation in growth during the first 6 months of postnatal life. They also pointed out that of all 3 anthropometric measures at 6 months, maternal and paternal stature impacted the most with maternal height having more effect on weight and head circumference. They concluded that the data highlight the importance of factors such as smoking and parity that can be manipulated by public health education and others such as gestational length that can be hopefully manipulated by careful prenatal care and attendance at prenatal clinics.

Hindmarsh PC, Geary MP, Rodeck CH, Kingdom JC, Cole TJ. Factors predicting ante- and postnatal growth. Pediatr Res. 2008;63:99-102.

Editor's Comment: *This interesting manuscript attempts to characterize factors that influence growth during infancy—and particularly at an age which is effected primarily by nutrition and for the most part is growth hormone independent. It is important to note that all of these data were collected from uncomplicated pregnancies. However, this large cohort is socioeconomically and anthropometrically representative of the UK population. Thus, the information is of extreme importance in obtaining a better understanding of how infants grow and which factors may be important. It is also important for pediatric endocrinologists to better understand the factors that may be contributing to growth failure in infants referred to their practices.*

William L. Clarke, MD

Height Sparing in Anorexia Nervosa?

Reports of height in girls with anorexia nervosa (AN) have conflicted between stunting and sparing. While undernutrition and low insulin-like growth factor (IGF)-I levels would be expected to stunt statural growth, high levels of growth hormone (GH), with its direct effects on the growth plate, and hypogonadism, resulting in delayed skeletal maturation, would be expected to preserve height.

Towards a better understanding, Prabhakaran et al compared 110 girls with AN (mean duration of illness 11.6 ± 13.2 months) to 98 age-matched controls (aged 12-18 years); 63 girls with AN and 79 controls were followed prospectively for one year. Girls were premenarcheal at baseline; 25 girls had AN and 10 were controls. At baseline, girls with AN had significantly lower BMI (mean 18.5 ± 2.1 vs 22.0 ± 3.2 kg/m^2, respectively), lower IGF-I levels (15.8% of girls with AN had IGF-I concentrations below the reference range and 52.6% had levels within the lowest quartile for pubertal stage, compared to 18.4% of the controls), and higher nadir GH levels on overnight sampling (mean 2.14 ± 1.17 vs 1.04 ± 1.01 ng/mL, respectively). Bone ages were similar, though the difference between bone age and chronologic age was lower by a few months in the AN group.

Midparental target heights (based on parental reports) and baseline heights were slightly higher for the AN group than controls (the latter 164.3 ± 6.9 vs 162.5 ± 6.5 cm, respectively). Height parameters did not differ significantly between the groups at 12-month follow up. Associations between nadir GH levels and z-scores for both height and predicted adult height (by Bayley-Pinneau method) were stronger in immature subjects and in controls. For girls with AN, these height parameters were associated with IGF-I levels instead, and inversely with duration of illness. The one-year increase in height z-score for immature girls with AN was predicted by baseline delay in bone age relative to chronologic age.

The authors concluded that hypogonadism (delayed skeletal maturation), not higher GH levels, preserves final height in girls with AN. The duration and severity of illness (and hence, IGF-I levels) also played an important role in height outcome.

Prabhakaran R, Misra M, Miller KK, et al. Determinants of height in adolescent girls with anorexia nervosa. Pediatrics. 2008;121: e1517-23.

Editor's Comment: *Endocrinologists are frequently consulted for the hypogonadism associated with AN. Patients' families present with the chief complaint of amenorrhea and raise concerns over bone mineralization.*[1] *While these are valid concerns, this paper supports the notion that treatment of the underlying disease process (ie, nutritional repletion and psychological correction of the distorted body image) is preferable to hormone replacement therapy, especially for girls who are still growing. The reader must keep in mind that the height outcomes found in this study are dependent on the duration and severity of illness and may not generalize to other groups.*

Adda Grimberg, MD

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Growth Plate Changes of Catch-up Growth Following Caloric Restriction: Morphologic and Gene Expression Changes, Especially HIF1α

To study the mechanism of catch-up growth, Even-Zohar et al examined growth plate morphology and gene expression in prepubertal male Sprague-Dawley rats subjected to 10 days of 40% caloric restriction. This degree of caloric reduction long-term had been shown to increase rat longevity, and was calculated based on the ad lib feeding of similar age and weight rats in a previous experiment (access to water remained unlimited). Rats were randomized into 3 groups: ad libitum (AL; unlimited food access throughout the experiment), food restricted (RES; 60% of the same chow throughout the experiment), and catch-up (CU; the 60% intake for 10 days followed by ad libitum feeding for the next 7 days).

Growth parameters confirmed the experimental design, though the catch-up growth was partial. Weight gain was 6.5 gm/day in the AL group, and only 1.2 gm/day in the RES group. The CU period augmented weight gain to 15.1 gm on the first day of ad lib feeding, followed by an average daily weight gain rate of 8% their total body weight compared to 4.5% in the AL group. Nonetheless, the CU group failed to completely regain their weight deficit by the end of the week. Similarly, humeral length was significantly reduced in the RES group throughout, and significantly improved at day 7 of CU but still shorter than the AL group.

The humeral epiphyseal growth plates (EGP) reflected the gross growth parameters. The EGP length (from the reserve zone to the ossification front of the metaphyseal bone) was constant in the AL group, shorter in the RES group, and showed progressive catch-up in the CU group. The average number of chondrocytes per column (proliferative zone through the last hypertrophic cell) was reduced in RES, and improved in the CU group from day 2 to 7 of ad lib feeding. The ratios of proliferative to hypertrophic zones were unaffected by the nutritional interventions.

Towards a mechanistic understanding, the EGPs were microdissected and total RNA was pooled from at least 15 sections from each zone of each animal to be studied by Affymetrix microarray. Between the RES and AL groups 4144 probes differed significantly. Interestingly, the gene expression profile of the CU group differed from RES by the first day of liberated feeding, yet remained different from AL for the remainder of the week. The investigators focused on genes with a so-called "up-down-up" (UDU) expression profile (highly expressed in AL, reduced in RES and increased again in CU). At least 2-fold changes were shown in 714 genes, going down from AL to RES and up from RES to CU; of these, 550 were differentially expressed among all 3 groups on the first day of refeeding. In silico analyses of functional groups and promoter of cluster revealed the UDU genes to be enriched for synthetic (macromolecule metabolism, RNA processing and translation, protein transport, secretion and degradation) rather than proliferative functions.

Of the 7 transcription factors whose downstream targets were enriched in the UDU gene list, hypoxia inducible factor (HIF)1α was selected by the investigators for further study. Messages of HIF1α and 3 of its target genes (one representing each function: glycolysis, proliferation/survival and chondrogenic/structural activity) were quantified by RT-PCR to validate the microarray findings. The UDU expression changes were specific to the growth plates, as hepatic expression of HIF1α did not differ among the 3 groups on the first day of liberated feeding. Immunohistochemistry of the proximal humeral EGPs showed HIF1α protein most intensely in the AL and CU proliferative zone cells, mainly in the nucleus (consistent with its function as a transcription factor); RES cells stained weakly for HIF1α. Microdissection and RT-PCR of tibial EGPs showed proliferative zone cells to exceed hypertrophic zone cells in HIF1α message levels as well; the 3-fold expression difference between the zones was constant across experimental conditions.

In summary, the authors found that nutritional

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restriction/refeeding has a significant effect on expression of HIF1α and its targets in the prepubertal rat EGP, and proposed that HIF1α plays an important role in regulating chondrogenesis. They further speculated on the mediators of HIF1α regulation by nutritional restriction/refeeding, including oxygen tension (presumably decreased in CU growth plates due to the increased EGP dimension and/or increased oxygen consumption, both of which can be expected from the rapid growth) and circulating hormone levels (such as insulin-like growth factor [IGF]-I, which is known to induce HIF1α and is itself regulated by nutritional status). One can easily see how this paper has opened the door for many follow-up studies, not just of HIF1α but of the other identified genes as well.

Even-Zohar N, Jacob J, Amariglio N, et al. Nutrition-induced catch-up growth increases hypoxia inducible factor 1 alpha RNA levels in the growth plate. Bone. 2008;42:505-15.

Editor's Comment: *This elegantly designed study marks an exciting new era of growth research. Historically, endocrinologists studied growth by focusing on changes in circulating hormone levels. Creation of the LID mouse (liver-specific IGF-I deficient via the cre-lox technique) shocked the traditional paradigm. The LID mice grew normally despite a 75% reduction in circulating levels of IGF-I, highlighting the surprising importance of autocrine/paracrine IGF-I for growth.[1] Thus, "the action" is now understood to be local, in the growth plates, rather than the circulation. Growth plate studies have increasingly permeated the growth literature, some of which have been reported in previous issues of GGH.[2,3] By applying technological advances, like the microarray and gene database analyses, to the newer growth-plate focus, this paper's study design has the power to not only spotlight previously expected "players" but identify new ones as well that are important for mediating growth and its various perturbations. Such experimental approaches are likely to herald accelerated advances in the growth field, though confirmatory evidence through alternative models are still required to validate the biologically significant findings and to rule out the potential for inter-species differences.*

Adda Grimberg, MD

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High Growth Rate of Girls with Precocious Puberty Exposed to Estrogenic Mycotoxins

Zeranol (α-ZAL; α-zearalanol) is an anabolic estrogen that has been used for increasing muscle mass in cattle and poultry. It is produced by Fusarium, and is thus a mycotoxin. Earlier studies have shown estrogenic mycotoxins in 5 of 36 girls with early thelarche in southeastern Hungary and a high incidence of central precocious puberty (CPP) in a northwest region of Tuscany (22 to 29 times higher than that in neighboring areas), suggesting an environmental estrogen exposure as causative. Zearalenone (ZEA) and its metabolites (ie, α-ZAL, β-zearalanol [β-ZAL], α-zearalenol [α-ZOL], and β-zearalenol [β-ZOL]) are apparently able to adopt a chemical configuration that resembles 17β-estradiol (E_2) and binds to estrogen receptors in target cells thus exerting estrogenic (agonist) action.

The aim of this study by Massart and colleagues was to test the hypothesis that environmental estrogenic exposure through mycotoxins could be associated with CPP in girls from the Viareggio countryside of northwest Tuscany, Italy. Thirty-two girls with CPP—defined as history of increased growth velocity, Tanner 2 breast development, and bone age advanced more than one year, LH and FSH responses to gonadotropin releasing hormone (GnRH) stimulation in the pubertal range, E_2 levels >25 pg/mL, and chronologic age ≤ 8 years—were studied. Group A comprised 17 girls came from the Viareggio countryside and group B were 15 girls from Pisa. In addition 31 age- and sex-matched control subjects from Viareggio (n=15, group C) and Pisa (n=16, group D) were studied as controls. Following diagnosis, all 32 girls with CPP were treated with triptorelin (TR) depot IM every 28 days for more than 12 months. Auxologic data and pubertal development were recorded initially and at 3- and 6-month intervals and bone age was done yearly and read by Greulich and Pyle method. Italian standards were used to determine height SDS, weight SDS, and height velocity SDS. Mycotoxin (ZEA, α-ZOL, β-ZOL, α-ZAL, and β-ZAL) levels were determined using high performance liquid chromatography from sera during the GnRH stimulation test at diagnosis and at 12 months of treatment.

All 63 girls were born at term and appropriate for gestational age. At the start of the study there were no significant differences between the CPP groups and the control groups. The only mycotoxins detected were ZEA and α-ZOL. At diagnosis 6 of the 17 girls (35%) at Viareggio had higher serum ZEA and α-ZOL levels than the other 3 groups; ZEA and its metabolites were not detected in the 15 CPP girls from Pisa or in the control subjects. All 32 girls with CPP (groups A and B) had undetectable mycotoxin levels after 12 months of GnRH agonist treatment. In order to study the differences between the girls who were mycotoxin positive versus those in whom mycotoxins were undetectable, 2 additional groups were formed. The 6 girls

who were mycotoxin positive (group E) were compared with the 26 girls who were mycotoxin negative (group F). At diagnosis there were no differences in chronologic age, target SDS in all 3 groups, and bone age and bone age/chronologic age ratio were not different in the CPP groups before and 12 months after treatment. However, group E and group F had different growth trends during treatment. Group E height SDS for chronologic age significantly increased from baseline during treatment while height SDS for chronologic age declined slightly in group F. Similarly, weight SDS for chronological age increased from baseline in group E, but not in group F. The BMI did not differ in groups with CPP at diagnosis or after 12 months of therapy. In addition, after 12 months of therapy, height SDS for bone age was higher in Group E than in Group F even though no difference was detected at the time of diagnosis. Height velocity SDS for chronologic age was also higher in group E than in group F and the control subjects, while height velocity SDS for chronologic age in group F was constant during treatment. At diagnosis serum ZEA levels correlated with height, SDS for chronologic age, weight SDS for chronologic age, and height SDS for bone age. There was no correlation detected for α-ZOL; at 12 months no correlations were detected. No differences were found in the groups with CPP at diagnosis and during treatment for LH, FSH, or for E_2.

The authors reviewed information known about ZEA as a non-steroidal mycotoxin produced by Fusarium species on several grains. Of note, beside the estrogenic activity, ZEA also has anabolic properties and ZEA food contamination could be either direct or indirect by carryover of mycotoxin in animal tissues such as milk and eggs after intake of contaminated feeds. In the US α-ZAL has been used widely as a growth promoter to fatten cattle. This application was banned in the EU in 1985. The ZEA metabolites mimic estrogens and act as estrogen receptor agonists; they have limited or no binding to carrier proteins. Thus they have easier access to estrogen target tissues and a potency that may be as much as 50 times greater than their actual concentrations suggest. The finding that the girls who were mycotoxin positive had a higher growth rate during TR treatment than those who were mycotoxin negative may be related to the anabolic effect of accumulated ZEA that persists despite effective GnRH agonist treatment. The authors referenced a publication that showed a prepubertal dose of estrogen replacement during TR treatment in girls with CPP is effective for at least 2 years in maintaining a height velocity of about +1 SDS without accelerating bone maturation.[1] Finally, the authors noted that although ZEA is stored in adipose depots, a single dose has a half-life of only about 22 hours in human blood. Thus incidental exposure may be time limited, but could induce a central maturation of the hypothalamic pituitary gonadal axis.

Massart F, Meucci V, Saggese G, Soldani G. High growth rate of girls with precocious puberty exposed to estrogenic mycotoxins. J Pediatr. 2008;152:690-5.

Editor's Comment: *There is a growing body of evidence concerning endocrine disruptors. Much of the information has been gathered from animal studies but there have been a few human studies that demonstrate a clear-cut association between such environmental agents and early puberty in children. Massart et al, in this carefully controlled study, demonstrated a significant association between an "endocrine disruptor" and CPP. The disruptor, ZEA and its metabolites, is a naturally occurring mycotoxin. Thus, the relationship between at least one estrogen disruptor and the occurrence of CPP in pediatric patients cannot be dismissed. This study should give encouragement to those who are attempting to identify other potential environmental contaminants that may be associated with endocrine disruption in the pediatric population and also should underscore the importance of taking a careful dietary and exposure history from each of our patients who present with similar clinical findings. It is disturbing that a derivative of this mycotoxin has been widely used as a growth promoter to fatten cattle in the US. Only with careful, well-documented information can these important associations be identified and exposures be limited or reduced.*

William L. Clarke, MD

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Long-term Follow-up of Idiopathic CPP Treated with GnRHa

Pasquino et al evaluated the impact of gonadotropin-releasing hormone analog (GnRHa) treatment on the adult height (AH), BMI, bone mineral density (BMD), and reproductive function of 87 girls with idiopathic central precocious puberty (ICPP). Patients were treated with depot triptorelin at a dose of 100 to 120 mcg/kg every 21 – 25 days for a period of 4.2 ± 1.6 years (range 3 – 7.9) and were then observed for 9.9 ± 2.0 years (range of 4 – 10.6) after discontinuing treatment; 32 untreated girls with ICPP served as controls. The AH of treated girls was 159.8 ± 5.3 cm, significantly higher than predicted adult height (PAH) with a gain in centimeters between PAH and AH of 5.1 ± 4.5. Although, on the whole, BMI increased, BMI SDS for chronological age was not different at the beginning or at the discontinuation of treatment, or years afterwards; patients who were overweight or obese at the beginning of treatment remained so by the end of therapy. Gonadotropin and estradiol levels decreased significantly with GnRHa therapy and rose above pre-treatment levels one year after discontinuation of therapy.

Ovarian volumes, were reduced during treatment and increased thereafter, while uterine length was unchanged during therapy and increased one year after discontinuing therapy. Menarche appeared at the age of 13.6 ± 1.1 years after withdrawal of GnRHa at 0.9 ± 0.4 years (range of 0.3 – 2.0); 82 patients had a pattern of regular menses, while 5 had oligomenorrhea due to intensive physical activity (which resolved when this activity was decreased), and 6 girls became pregnant and delivered normal offspring. The BMD calculated both by area and volume were decreased at discontinuation of therapy when compared to controls, but after complete resumption of gonadal activity were not significantly different from controls. The authors concluded that GnRHa treatment of girls with ICCP is safe for the reproductive system, BMD, and BMI and is helpful in reaching an AH close to target height.

Pasquino AM, Pucarelli I, Accardo F, Demiraj V, Segni M, Di Nardo R. Long-term observation of 87 girls with idiopathic central precocious puberty treated with gonadotropin-releasing hormone analogs: Impact on adult height, body mass index, bone mineral content and reproductive function. J Clin Endocrinol Metab. 2008;93:190-5.

Editor's Comment: *The long-term follow-up by Pasquino and colleagues of a large cohort of girls with ICPP treated with GnRHa, suggests that this form of therapy is safe—leading to a normal resumption of gonadal function one year after discontinuation of therapy. This was manifested by an increase of gonadotropins and estradiol to normal levels for age, an increase in ovarian and uterine dimensions, appearance of menarche at a mean of 0.9 ± 0.4 years after discontinuation of treatment with the maintenance of a normal menstrual pattern thereafter, and with normal pregnancies and deliveries of healthy offspring in 6 girls. This, as well as previous data1 should assure physicians and parents of the safety of this medication in regard to gonadal function and the future reproductive health of girls with ICPP treated for prolonged periods with GnRHa. Recent data suggest that children with ICPP may have an increased BMI and that GnRHa treatment might contribute to the worsening of this parameter.[2] Although, as a whole, BMI increased during therapy, it remained in the same centile or SDS throughout treatment and patients who were already obese or overweight at the beginning, remained so at the end of treatment. There is still controversy regarding the beneficial effect of GnRHa treatment on the AH of treated girls with ICPP.[1,2] In this study AH of treated girls was significantly increased when compared to PAH before the beginning of therapy and, as a whole, patients reached or overcame their TH. When the AH height of untreated control subjects was compared to that of treated individuals it was found to be about 5 cm shorter—more than 4 cm below their TH and with no significant gain over their PAH. However, while GnRHa therapy seems helpful in reaching an AH close to TH, it is clear that there is a marked variability in individual response. Another worrisome issue is the effect of suppression of ovarian activity on BMD, both during therapy and long-term.[3] Even though this study demonstrated a decrease in bone accretion during GnRHa therapy, bone mineral density calculated both by area and volume seemed to normalize after the complete resumption of ovarian activity and peak bone mass was reached. Although GnRHa therapy has been widely used in the treatment of girls with ICPP for the last 20 years, many doubts in regard to its long-term benefits and safety remain. This long-term follow-up of a large cohort of treated girls may ease concerns.*

Roberto Lanes, MD

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Genetics of Dwarfism

The centrosome is a cytoplasmic organelle that prepares the mitotic spindle for chromosome segregation and also regulates progression of the cell cycle through mitosis.[1] Pericentrin 2 (PCNT2) is a centrosomal protein that is essential for the integrity of the mitotic spindle as it links the microtubules of the mitotic spindle apparatus to the centrosomal core. PCNT2 is also involved in the process of normal cell division at the G2-M checkpoint. Thus, loss of PCNT2 likely results in cell death because of defects in both chromosome segregation and mitosis. Rauch et al and Griffith et al have described clinical syndromes associated with biallelic loss-of-function mutations in the gene encoding PCNT2—also termed kendrin (*PCNT2* - chromosome 21q22.3-qter - OMIM 605925).

Microcephalic osteodysplastic primordial dwarfism (MOPD) is characterized by intrauterine and postnatal growth retardation, short limbs (brachymelia), and microcephaly (Figure 1). The humeri and femora are broad, shortened, and bowed. Clinically, MOPD has been subclassified into types I (OMIM 210710), II (OMIM 210720), and III (OMIM 210730). Types I and III are considered to be variations of the same disorder and are associated with dysplasia of the skull, vertebrae, and limbs and malformations of the brain and early death; no gene mutation has as yet been identified in these subjects. In patients with MOPD II, an autosomal recessive disorder, facial features are similar to those of patients with Seckel syndrome (*vide infra*); birth weight is <1.5 kg at term;

average adult height is 100 cm; adult head circumference is 40 cm, mentation is reasonably normal. Adults with MOPD II have a shortened life-span because they are at increased risk for development of type 2 diabetes mellitus, obesity, and cerebrovascular accidents. MOPD II is not considered a syndrome of premature aging as these patients are not at risk for development of neoplasia nor do their chromosomal telomeres display an accelerated rate of shortening. Utilizing the families of patients with MOPD II born to consanguineous parents and genome wide linkage analysis, Rauch et al localized this disorder to chromosome 21q22.3—the site of *PCNT2*. After analysis of the 47 exons of *PCNT2* in 25 unrelated patients with MOPD II, these investigators identified 29 distinct null mutations (12 stop and 17 frameshift) scattered through the gene. Interestingly, in patients with MOPD II, *PCNT2* is transcribed (mRNA levels are normal or slightly decreased) but not translated (PCNT2 protein levels are absent or low), as its mRNA is subjected to nonsense-mediated mRNA decay directed by pretranslational mRNA surveillance mechanisms. Heterozygous ($PCNT2^{+/-}$) parents synthesize less PCNT2 protein than normal subjects and are reported to have significantly short adult stature raising the possibility that variants of *PCNT2* are involved in determination of stature in the normal population.

Seckel syndrome is also a heterogeneous, autosomal recessive disorder that has been subclassified into types 1 through 4 depending on linkage to different chromosomal regions (3q22, 18p11, 14q, 21q22.3). It is characterized by *symmetrical* prenatal and postnatal growth retardation, microcephaly with developmental delay, and "bird-like" facial features (small head, large eyes, beak-like nose, micrognathia). The clinical characteristics of patients with Seckel syndrome type 4 (chromosome 21q22.3-qter; OMIM 611860) are similar to those of patients with other subtypes. Griffith et al, utilizing a genome wide association procedure in 2 consanguineous families with Seckel syndrome members, also localized the disorder to chromosome 21q22.3 and identified homozygous inactivating (nonsense, single base pair deletion or insertion) mutations in *PCNT2* in affected patients.

The reason that loss-of-function mutations in *PCNT2* result in 2 clinically similar (microcephaly, facial features, growth retardation) but distinct (proportionate versus non-

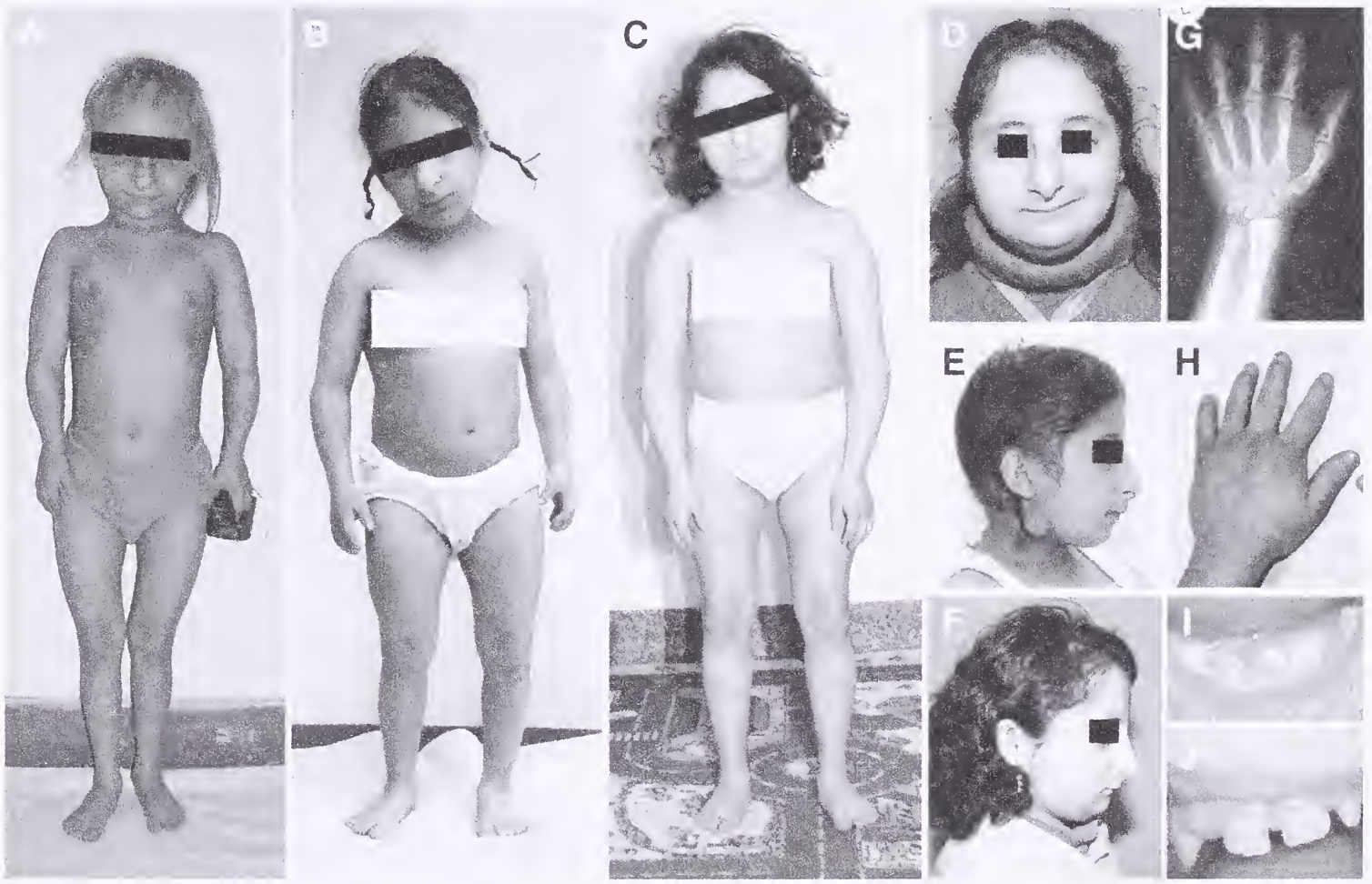

Figure 1. Phenotype of MOPD II patients. (A) P18 at age 8 years 3 months with a height of 84 cm corresponding to a normal size for a female infant aged 1 year 3 months; (B and E) P1 at age 8 years 8 months with a height of 85 cm; (C and F) P2 at age 12 years 6 months with a height of 95 cm and at age 14 years with a height of 96 cm (D) corresponding to a normal size for a female aged 3 years. Note short lower arms especially in P18, mild truncal obesity and premature puberty in P1, significant facial asymmetry in P2 (D), and absence of a sloping forehead typical of microcephaly syndromes. All three patients demonstrate a long nose with prominent tip and hypoplastic alae and small mandible described as typical for patients with MOPD II. (G and H) X-ray and an image of the dorsum of the left hand of patient P2 showing generalized brachydactyly with diaphyseal constriction (overmodeling) of metacarpals and phalanges, as well as abnormal flat shape of the distal radius and ulna epiphyses. (I and J) Hypoplasia and partial agenesis of teeth from patient P2, enamel hypoplasia in teeth from patient P18.

symmetrical short stature, reasonably normal mentation versus developmental delay) disorders of MOPD II or Seckel syndrome is uncertain. It has been suggested that in MOPD II, the *PCNT2* mutations may adversely affect function of the centrosome, while in Seckel syndrome the mutations may impair mitotic progression.[1]

Rauch A, Thiel CT, Schindler D, et al. Mutations in the pericentrin (PCNT) gene cause primordial dwarfism. Science. 2008;319:816-9.

Griffith E, Walker S, Martin C-A, et al. Mutations in pericentrin cause Seckel syndrome with defective ATR-dependent DNA damage signaling. Nat Genet. 2008;40:232-6.

First Editor's Comment: *Seckel syndrome type 1 has been ascribed to inactivating mutations in the DNA damage detection and repair ataxia-telangiectasia and Rad3-related gene (ATR; chromosome 3q22-24, OMIM 601205). Inactivation of PCNT2 adversely affects function of ATR protein-dependent effects on monitoring of the cell cycle; PCNT2 acts at a point downstream of ATR. Mutations in several genes that encode centrosomal and mitotic spindle-related proteins have been associated with isolated primary microcephaly with normal stature (CDK5RAP2, ASPM, MCPH6) and primary microcephaly with short stature (MCPH1). Homo floresiensis is species of hominids whose fossils have been found in Indonesia and who have several features in common with MOPD II including an adult height of 100 cm, small brain but normal intelligence, and skeletal anomalies raising speculation that they may have been humans with MOPD II or defects elsewhere in the DNA damage-repair pathways.*

The findings in patients with MOPD II and Seckel syndrome may be compared with those of Hutchinson-Gilford progeria,[2,3] a syndrome of premature aging due to a monoallelic mutation in the gene (LMNA) encoding lamin A. Progeric subjects are characterized by postnatal growth retardation, small head circumference, abnormalities of the skin (altered pigmentation, sclerosis, alopecia), hypodontia, lipodystrophy, restricted joint mobility, cardiovascular abnormalities, and early death. Lamin A (chromosome 1q21.2, OMIM 150330) is an essential component of the protein network found within the nuclear membrane. Ninety percent of patients have a C-to-T substitution at nucleotide 1824 resulting in substitution at codon 608 of glycine GGC to glycine GGT. This nucleotide change activates a cryptic splice donor site that removes 150 nucleotides from transcribed LMNA mRNA. The translated protein retains farnesyl groups that link mutant and wt lamin A molecules and prevents their release from the inner nuclear membrane, thereby interfering with cell mitosis and gene expression. Experimentally, prevention of farnesyl attachment to mutated lamin A allows the protein to separate from the inner nuclear membrane. A drug that inhibits farnesyl transferase ameliorates a mouse model of progeria. An open-label trial of this agent is now underway in progeric patients.

Allen W. Root, MD

Second Editor's Comment: *Seckel syndrome refers to a genetically heterogenous group of autosomal recessive conditions (SCKL1-4) characterized by severe pre- and postnatal growth deficiency and marked microcephaly. While all 4 conditions have been chromosomally mapped, the gene locus is known only for SCKL1; it encodes ATR, which functions to coordinate cellular responses to DNA damage. More specifically, ATR signaling responds to*

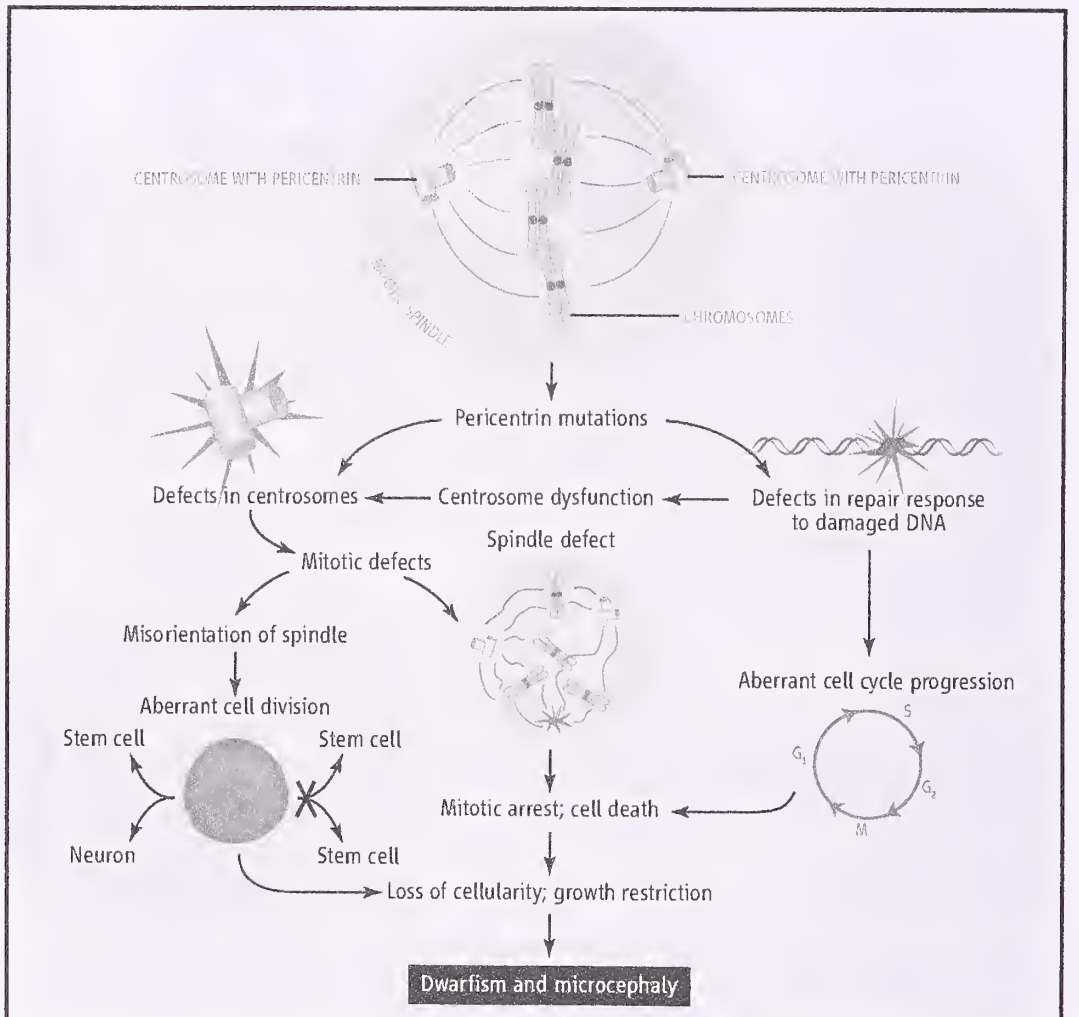

Figure 2. Pericentrin function in dwarfism with microcephaly. Centrosome defects, disrupted DNA damage pathway, and impaired cell division are potential contributors to cellular loss and growth restriction that characterize human dwarfism with microcephaly.

single-stranded DNA damage.

Griffith and colleagues carried out an SNP-microarray genome-wide scan to detect regions of homozygosity on 2 consanguineous families with individuals clinically diagnosed with Seckel syndrome and showing evidence of defective ATR signaling. The scan identified a region on chromosome 21q22.3, which corresponds to the SCKL4 locus, that contained the gene encoding the centrosomal protein, PCNT. Pericentrin was considered a candidate because mutations in other centrisomal protein genes were known to cause primary microcephaly (Figure 2). Genomic sequencing revealed a homozygous nonsense mutation in exon 4 in affected members of one family and a homozygous single basepair deletion in the other; both were predicted to result in loss of function. A similar PCNT mutation was detected in a third patient with typical features of Seckel syndrome.

Pericentrin localizes in cells to the pericentriolar material where it is believed to interact with several structural centrosomal proteins involved in the attachment of microtubules during mitotic spindle formation. It also appears to act as a scaffold to recruit signaling molecules, such as protein kinase A (PKA) to centrosomes. The authors carried out a number of experiments to document the absence of centrosomal pericentrin in patient cells. They also induced DNA damage with UV light and showed that ATR-dependent DNA damage response signaling that is normally activated during the cell cycle was defective similar to that observed in cells from patients with SCKL1 (Figure 3). This step is often referred to as G2-M checkpoint arrest, a process that prevents cells from entering into the M (mitotic) phase of the cell cycle with damaged DNA. The authors postulated that pericentrin deficiency interferes with growth through a general impairment in the progression of cells through mitosis. They also noted that identification of pericentrin mutations provides an interesting convergence between microcephaly genes implicated in ATR signaling and those involved in centrosomal function. It makes sense that the profound growth deficiency of Seckel syndrome is due to a disturbance in the machinery that directs cell division, but it would have been impossible to predict the specific defect without recent advances in genomic technology.

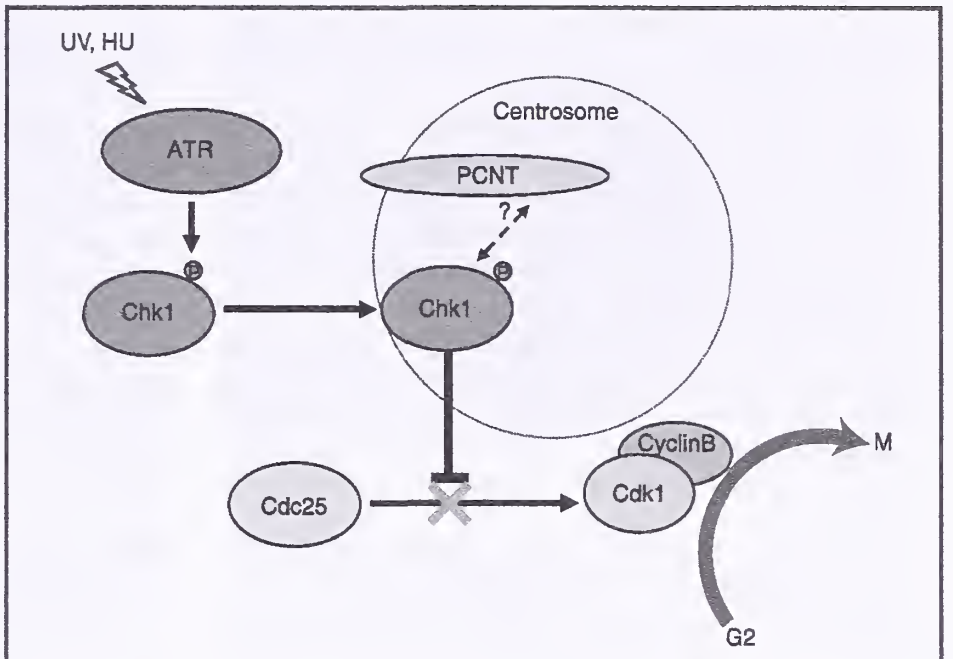

Figure 3. Model of pericentrin's role in ATR-dependent G2-M checkpoint arrest. Low-dose UV and hydroxyurea activate ATR kinase, which then phosphorylates downstream targets including Chk1 kinase. Activating phosphorylation is required for accumulation of Chk1 at the centrosome after DNA damage. Direct or indirect binding of Chk1 by pericentrin could mediate localization of Chk1 to the centrosome. Chk1 localization at the centrosome inhibits Cdc25, preventing cyclin B/Cdk1 activation and thus the transition from G2 to mitosis.

William A. Horton, MD

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Genetics Influences Allelic Expression Patterns

Maternal and paternal alleles of autosomal genes were historically assumed to be expressed at the same levels. However, recent observations suggest that their expression may differ, ie, differential allelic expression (DAE), and that this difference may contribute to variability of clinical phenotypes in dominantly inherited disorders. To determine if there is a genetic component to DAE, Cheung et al examined patterns of allele expression in monozygotic twins.

The authors studied lymphoblastoid B cells from 21 monozygotic twins and 10 unrelated individuals. They took advantage of single nucleotide polymorphisms (SNPs) that map to exons so that they could be detected in mRNA and identified 285 instances in which "A" and "B" alleles could be distinguished in the respective mRNA transcripts. To determine the extent of differential expression of these alleles regardless of twinning, they first examined DAE in one member of each twin pair and the unrelated individuals using deviation from equal expression of the 2 alleles as a measure of DAE. Deviation was considered

nominally statistically significant for half of the allele pairs, and 17% displayed an expression difference of 2-fold or more for one allele over the other.

Next they searched for DAE in the monozygotic twins utilizing 211 SNPs that were found to be heterozygous in 5 or more twin pairs and did an analysis of variance to determine the significance of twin resemblance. The results revealed much greater similarity between twins than predicted by chance. In a few instances in which more than one informative SNPs mapped to the same gene, the results were concordant. Twin resemblance for DAE was detected not only for genes whose alleles deviated substantially from equal expression, but also for genes whose alleles are expressed at relatively similar levels.

The authors drew 2 conclusions from their results. First, at least 50% of genes expressed in lymphoblastoid B cells show some degree of DAE. The difference is greater than 2-fold for some genes. Second, much of the observed DAE seems to be under genetic control.

Cheung VG, Bruzel A, Burdick JT, Morley M, Devlin JL, Spielman RS. Monozyogotic twins reveal germline contribution to allelic expression differences. Am J Hum Genet. 2008; 82:1357-60.

Editor's Comment: *This investigation provides another explanation for why monozyogotic twins are so similar. A paper was recently reviewed in GGH[1,2] suggesting that patterns of epigenetic modification diverge in monozygotic twins as they age. Since epigenetic modification influences expression of genes, one wonders if DAE varies with age or correlates at all with such modifications. Similarly, it would be interesting to know the extent to which DAE occurs in cell types other than lymphoblastoid B cells.*

William A. Horton, MD

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Growth Hormone Therapy Improves Mental and Motor Development in Young Prader-Willi Patients

Prader-Willi syndrome (PWS) is increasingly diagnosed in early infancy because pediatricians and neonatologists are more aware of the clinical picture (muscular hypotonia, feeding difficulties, failure to thrive, and psychomotor delay). The genetic cause of PWS is an alteration in the long arm of paternal chromosome 15 (by deletion, microdeletion, maternal uniparental disomy, mutation of imprinting centre, chromosomal rearrangement). It is well known that methylation analysis is an efficient tool for early and reliable diagnosis of PWS. Children with PWS have an abnormal body composition with a relatively high body fat percentage and a low lean body mass (LBM). Even in PWS infants who are underweight, body fat percentage is high.

Treatment with human growth hormone (hGH) in older children with PWS results not only in an increased growth response but also in an improvement in body composition, with a decline in fat percentage and an increment in LBM, resulting in increased muscle strength and agility. The effects of hGH therapy on psychosocial development in PWS have not been well studied.

Festen and colleagues evaluated psychomotor development in PWS infants and toddlers during hGH treatment compared to controls. Forty-three PWS infants were evaluated at baseline; 29 of them were randomized into a GH group (n=15) receiving 1 mg/m^2/day of GH or a non-GH-treated control group (n= 14). At baseline, and after 12 months of GH treatment, an analysis with Bayley Scales of Infant Development II (BSID-II) was performed. Data were converted to percentage of expected development for age, and changes during follow-up were calculated.

Infants in the GH group had a median age of 2.3 years (interquartile range [IQR] 1.7–3.0) and the median age of the control group was 1.5 years (IQR 1.2–2.7) ($p=0.17$). Both mental and motor development improved significantly during the first year of study in the GH group vs the control group: median (IQR) change was +9·3% (–5.3 to 13.3) vs –2.9% (–8.1 to 4.9) ($p<0.05$) in mental development and +11.2% (–4.9 to 22.5) vs –18.5% (–27.9 to 1.8) ($p< 0.05$) in motor development, respectively. Thus, one year of hGH treatment significantly improved mental and motor development in PWS infants compared to controls. Infants with lower developmental age had the greatest improvement in motor development. There was also a normalization of head circumference and a significant increase in height SDS in the GH group, but not in the control group after one year of hGH treatment. The hGH was well tolerated; compared to randomized controls, hGH did not induce disadvantageous effects on sleep-related breathing disorders, carbohydrate metabolism and thyroid hormone levels.

Festen DAM, Wevers M, Lindgren AC, et al. Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader–Willi syndrome. Clin Endocrinol. 2008;68:919-25.

Editor's Comment: *The best point of time to initiate hGH therapy for PWS remains unknown. Eiholzer et al[1] do not recommend starting hGH therapy in PWS in the first year of life because of an increased risk of sudden infant death during this period. Festen and colleagues evaluated whether hGH treatment started at an early age could contribute to an improvement in mental and motor development in a group of PWS patients. They*

found a significant improvement of both mental and motor development in the GH group compared to the control group. Children with lower developmental age had the greatest improvement in motor development, suggesting that hGH treatment might be considered at an early developmental age to optimize the hGH effects on motor development. They also found that hGH did not induce disadvantageous effects on sleep-related breathing disorders.

In their study, insulin-like growth factor (IGF)-I levels increased rapidly during hGH treatment from below the normal range to the high-normal range. IGF-I receptors have been localized in several areas in the human brain, indicating that IGF-I may have a neuroregulatory role in the central nervous system. Theoretically, IGF-I may directly influence the central nervous system or hGH might induce local IGF-I expression in brain tissue, thereby improving psychomotor development. Another possible explanation for the improvement in mental development during hGH treatment might be that, because of the improved motor development, children are able to sit, stand and walk independently, enabling them to explore and interact with the environment and resulting in a subsequent improvement in mental development. The results of this study suggest that early start with hGH might be beneficial in PWS. However, long-term double-blind studies are needed to evaluate the efficacy and safety of the early treatment with hGH on cognition in childhood and adulthood.

Yoshikazu Nishi, MD

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Central Adrenal Insufficiency, Pituitary and Neuroradiological Alterations in Prader-Willi

Prader-Willi syndrome (PWS; OMIM 176270) is a genetic disorder caused by an alteration in the long arm of paternal chromosome 15 (by deletion, microdeletion, maternal uniparental disomy, mutation of imprinting centre, chromosomal rearrangement). PWS is characterized by a complex clinical picture (short stature, uncontrollable hyperphagia, obesity, hypogonadism) and growth hormone deficiency that seem to be a central hypothalamic/pituitary dysfunction.

The annual death rate of PWS patients is very high (3%). Many of these deaths are sudden and unexplained. Because most deaths occur during infections and PWS patients suffer from various hypothalamic insufficiencies, de Lind van Wijngaarden and colleagues investigated whether PWS patients suffer from central adrenal insufficiency (CAI) during stressful conditions. Twenty-five children genetically confirmed PWS were randomly selected. Twelve patients had paternal deletion (63%), 6 had maternal disomy (32%), and one an imprinting center mutation (5%). Median age of patients with PWS was 9.7 years (range 3.7 to 18.6 years). All were treated with recombinant human growth hormone (rhGH). Overnight single-dose metyrapone tests were performed. Metyrapone (30 mg/kg) was administered at 2330 h. At 0400, 0600, and 0730 h, ACTH, 11-deoxycortisol, cortisol, and glucose levels were measured. Diurnal salivary cortisol profiles were also assessed on a different day at wake-up, 30 minutes after wake-up, at 1400 h, and at 2000 h. Fifteen patients (60%) showed an insufficient ACTH response at the metyrapone test. There was no significant difference in age, gender, genotype, and BMI SD score between patients with CAI and those without. Morning salivary cortisol levels and diurnal profiles were normal in all children, suggesting that CAI becomes apparent only during stressful conditions.

Moreover, Iughetti and colleagues retrospectively analyzed 91 patients with PWS (42 females, 49 males; age range 0.7 to 16.8 years) by cerebral MRI to determine whether there was any diminution in the anterior pituitary gland or other neuroradiological alterations. All subjects were genetically confirmed as PWS (58 microdeletions, 8 deletions, 28 maternal uniparental disomy). Of these 91 patients, MRI analysis showed a reduction in pituitary height (height <1 SD) in 45 patients (49.4%: 23 cases <2 SD; 20 males, 25 females) with 4 cases of empty sella, a complete absence of the posterior pituitary bright spot in 6 patients (6.6%) and other neuroradiological alterations in 10 patients (11%: 8 cases of ventricular enlargement, 2 cases of thin corpus callosum). Altogether, neuroradiological alterations were present in 61 of the 91 (67%) patients. No genotype-phenotype relationship was shown. These results of both de Lind van Wijngaarden and Iughetti indicate that CAI and neuroradiological alterations are more frequent in PWS patients than has been reported to date.

de Lind van Wijngaarden RF, Otten BJ, Festen DAM, et al. High prevalence of central adrenal insufficiency in patients with Prader-Willi syndrome. J Clin Endocrinol Metab. 2008;93:1649-54.

Iughetti L, Bosio L, Corrias A, et al. Pituitary height and neuroradiological alterations in patients with Prader-Labhart-Willi syndrome. Eur J Pediatr. 2008;167:701-2.

Editor's Comment: *These are very interesting observational studies, which provide important information for physicians who care for those with PWS. Strikingly, de Lind van Wijngaarden and colleagues reported 60% of PWS patients had CAI; the high percentage of CAI in PWS patients might explain the high rate of sudden death in these patients, particularly during infection-related stress. Because metyrapone blocks cortisol synthesis, it causes a sudden increased demand for ACTH production, a*

situation mimicking stress. Patients with an insufficient ACTH response during the metyrapone test are therefore considered as having CAI during stressful conditions such as infection and surgery. In view of the importance of an adequate function of the hypothalamus-pituitary-adrenal axis for survival, the high prevalence of CAI may be an explanation for the high death rate in PWS patients. In addition to CAI, the condition of acutely ill PWS patients is further compromised by an increase in those with sleep apnea and sudden death during upper respiratory infection. Therefore, de Lind van Wijngaarden and colleagues stated that PWS patients should be considered to have CAI during stress until proven otherwise with a metyrapone test and they recommended hydrocortisone treatment for PWS patients during stressful conditions including mild upper respiratory infections.

From these results, both neuroradiological alterations and CAI may relate mutually and may be important risk factors for a tendency of sudden, unexpected death in PWS patients. Further studies, including functional and longitudinal neuroradiological investigation, are needed to clarify these problems in PWS patients.

Yoshikazu Nishi, MD

Genital Function and Sensitivity Following Feminizing Surgery

Like other disorders of sex development (DSD), congenital adrenal hyperplasia (CAH) in 46,XX can be associated with ambiguous genitalia at birth. Clinical management commonly involves surgery performed during infancy and childhood to feminize the appearance of genitals. However, it has been suggested that surgery to the clitoris potentially disrupts neurological pathways and compromises erotic sensation and pleasure. In a cross-sectional investigation, Crouch and colleagues investigated the genital sensitivity of women with CAH and 10 healthy controls (23 to 38 years old). Sensitivity thresholds for the clitoris and upper vagina were measured using a GenitoSensory Analyzer and sexual function by standardized self-report questionnaire including 7 subscales assessing sexual anorgasmia, satisfaction, sensuality, communication, vaginal penetration difficulties, frequency of intercourse and avoidance. Thirty-two of 56 eligible women with CAH (17 to 39 years of age) agreed to participate: 25 with classic CAH, 4 with non-salt losing CAH, and 3 with late-diagnosed CAH. A total of 28 of 32 women participated in sensory testing, including 4 who had not undergone prior genital surgery. The sample is heterogeneous with regard to the type of genital surgery (clitoridectomy versus clitoral reduction and with or without surgery to the lower vagina), age at surgical procedures, and number of surgical procedures.

Clitoral sensation (temperature) testing indicated relative impairment for those who underwent clitoridectomy. As anticipated, clitoral sensation was not impaired in those with CAH who had not undergone surgery. In comparison with control group participants, women who had undergone clitoral reduction had a higher median threshold for warmth detection and a lower median threshold for cold. Vaginal sensitivity (vibratory) testing could not be assessed in some participants due to introital vaginal stenosis which prevented insertion of the vaginal probe. In addition, some control group participants chose not to undergo vaginal testing. For those who did, no difference was observed in vaginal sensation between the CAH group and control group participants (regardless of prior vaginal surgery).

Assessment of sexual function also proved to be challenging in this study; only 19 of 32 CAH participants adequately completed the questionnaire because of

***GRISS* sexual function scores in women with CAH** divided into those with and without surgery compared to normal controls

	Median CAH (range)		Median Normal	P Value
	Surgery	No Surgery	(range)	(Kruskal-Wallis test)
No. pts	15*	4	10	
Global score	5 (1-9)	4 (1-5)	2 (1-8)	0.029
Infrequent intercourse	8 (1-9)	6 (3-8)	5 (1-7)	0.030
Non communication	4 (1-9)	5 (2-6)	5 (3-9)	0.884
Disatisfactioin	4 (1-9)	3 (2-5)	3 (1-6)	0.195
Avoidance	6 (1-9)	5 (4-7)	2 (1-7)	0.043
Non sensuality	5 (1-8)	3 (2-6)	2 (1-9)	0.331
Vaginal penetration difficulties	6 (1-9)	1 (1-2)	1 (1-2)	0.006
anorgasmia	6 (3-9)	4 (3-6)	3 (2-9)	0.065

Score range 1 to 9 with 5 or greater indicating difficulty.

*One respondent excluded since she did not indicate a history of surgery.

lack of sexual activity upon which responses depended. Those women with CAH who had undergone surgery reported worse scores on the intercourse frequency, vaginal penetration difficulties, and anorgasmia ($p=0.065$) subscales compared with healthy controls and women with CAH who had not undergone surgery. Scores on global sexual dysfunction and avoidance were similar in women with CAH with and without surgery (Table). Significant correlations were detected between self-reported global sexual dysfunction and clitoral sensitivity impairment (Figure). The authors concluded that surgery is associated with a loss of sensitivity, and that impaired clitoral sensitivity is a result of surgical damage to the innervation of the clitoris. The authors further concluded that surgery is associated with sexual difficulties, citing a moderate but significant linear relationship between impaired clitoral sensitivity and the severity of sexual difficulties.

Crouch NS, Liao LM, Woodhouse CRJ, Conway GS, Creighton SM. Sexual function and genital sensitivity following feminizing genitoplasty for congenital adrenal hyperplasia. J Urol. 2008;179:634-8.

Editor's Comment: *These findings support previous research[1,2] suggesting an association between sexual dissatisfaction and genital surgery. This study goes beyond earlier research, however, by providing evidence for a mechanism mediating this association. Several challenges to interpretation of these data are worthy of note. Detailed operative records were available for only 15 women. This number is too small to enable comparisons of the effects of different surgical techniques. In addition, a significant number of women were unable to undergo vaginal testing due to proximal vaginal stenosis. On one hand, this difficulty provides important information, as the participants had reportedly undergone previous vaginoplasty to overcome penetration difficulties, yet penetration challenges clearly remain. A similar problem was seen in the effort to assess sexual function. Nineteen of 32 participants were unable to complete the questionnaire, citing lack of sexual experience necessary to complete questionnaire items.*

It can be argued that clitoridectomy is rarely performed these days, and as such, numbness associated with this operation does not apply to the types of techniques currently performed. However, the authors indicated that only a third of the women in their sample who had undergone a more conservative technique, clitoral reduction, reported normal clitoral sensitivity. Most participants in this study underwent surgery in the early 1980s. The authors indicated that most of the clitoral procedures these participants experienced were based on the dorsal neurovascular bundle preservation approach described in 1981 by Mollard,[3] a procedure the authors noted that has been widely used after 1985 and which has become the basis of current practice.

If the findings from this study are replicated in future studies, important issues need be carefully considered. Genital feminizing surgery for patients with CAH is typically performed prior to the age of consent. It is an unanswered question whether most women with CAH would knowingly sacrifice genital function for appearance. The difficult decision of opting for surgery (or not) is often left to parents/caregivers. As such, at the time of consent, they should be armed with clear information relating to the possibility of impairment of genital sensitivity and function. As noted by the authors, "informed consent should be based not just on the technical aspects of surgery and risks, but on a developed understanding and appreciation of potential implications for future sexual lives."

David E. Sandberg, PhD

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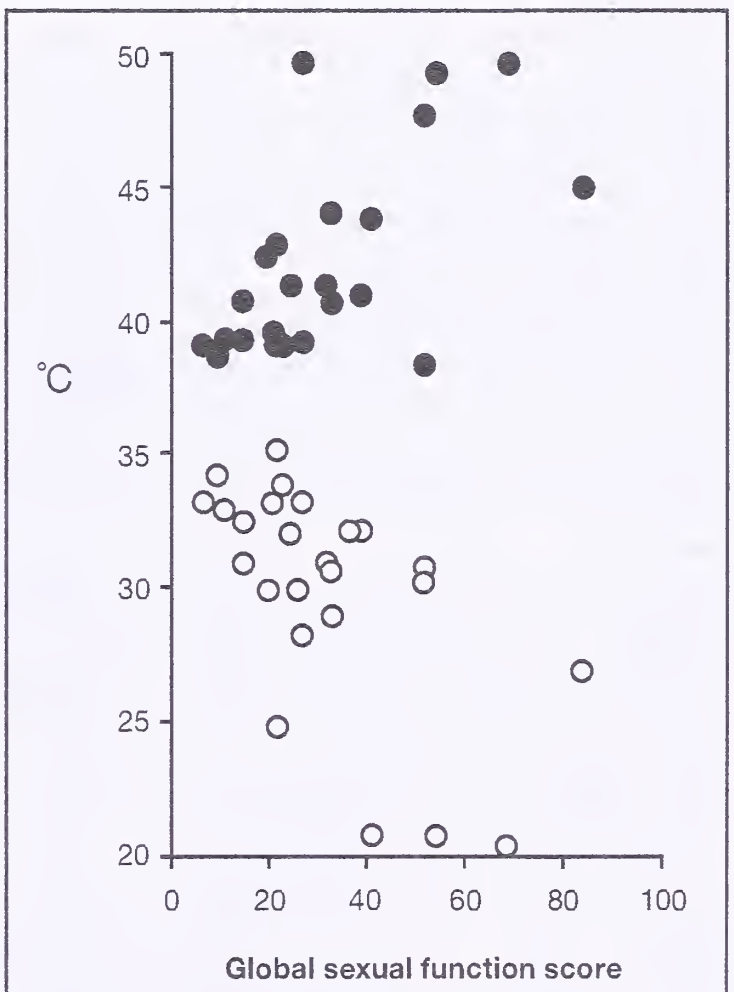

Threshold warm (filled circles) and cold (open circles) sensation of C of clitoris in women with CAH in relation to global sexual function score (Spearman's correlation coefficient r = 0.55, p = 0.005 and r = – 0.64, p = 0.001, respectively).

Diagnosis of Congenital Central Hypothyroidism in Infants

In the Netherlands, since 1995, a primary thyroxin (T_4) determination with supplemental thyroid-stimulating hormone (TSH) and T_4-binding globulin (TBG) measurements have been used as a routine screening protocol for congenital hypothyroidism. This screening approach was developed as congenital hypothyroidism of central origin (CH-C)—often complicated by hypoglycemia due to growth hormone deficiency and/or ACTH deficiency—poses an additional threat to the central nervous system development. The authors considered that a rapid diagnosis is critical in this population. The neonatal CH screening program was therefore adapted to improve detection of TSH deficiency. Indeed in a recent evaluation of the nationwide prospective screening program from 1995-2000 an increase of 1/16404 of CH-C was demonstrated with a detection rate of 91.6%. From these data CH-C—formerly considered as a rare entity—would make up 13.5% of all cases of permanent CH detected in a 6-year study period. The results of this showed that the TRH test plays a pivotal role in young infants.

Infants with neonatal screening results indicative of CH-C and subsequent free T_4 <0.93 ng/dL and TSH <15 µU/mL were enrolled in the study; 26 out of 385,042 neonates met the criteria and were tested within 3 months of birth. A TRH test was performed on 21 subjects; 6 of these children were found to have false-positive screening results. The remaining 15 infants were found to have CH-C during a 5-year follow-up. In this group cortisol deficiency was present in 9 cases, GHD in 10 cases, and gonadotropins deficiency in 6 subjects. TRH tests were interpreted by plotting results at several times after TRH administration. On the basis of former studies an adequate TSH response to TRH was characterized by a peak concentration greater than 15µU/mL and a return to baseline within 3 hours. In response to TRH, patients showed either diminished increase (type 2 response) or slightly delayed but excessive increase and delayed decrease of plasma TSH (type 3 response). All patients with type 3 TSH response had multiple pituitary hormone deficiencies (MPHD), whereas the majority of patients (67%) with type 2 response—which reflects an impaired TSH secretion—had isolated TSH deficiency. In 12 of 15 infants, the screening test provided the first indication of CH-C. The most frequently encountered problems were pathological neonatal jaundice (40%), hypoglycemia (33%), and persistent vomiting (20%). Fourteen children underwent MRI of the brain, 8 had posterior pituitary ectopia (PPE). All of these patients had MPHD.

The TRH test, in spite of the difficulties in establishing the pattern of a normal response in relation to age, appears to be crucial in the diagnosis of CH-C. It allows immediate assessment of the hypothalamic-pituitary function, and therefore rapid and appropriate treatment may be given. This appears to be particularly relevant for the group of infants screened in the early neonatal period as presenting at the typical TSH response: first increased and thereafter with a delayed return to normal.

van Tijn DA, de Vijlder JJ, Vulsma T. Role of the thyrotropin-releasing hormone stimulation test in diagnosis of congenital central hypothyroidism in infants. J Clin Endocrinol Metab. 2008;93:410-9.

Editor's Comment: *A frequently used strategy for the diagnosis of CH is to first measure T_4 in all samples, followed by TSH measurement for samples with low T_4 values. Several North American states use this strategy. In the Netherlands, the latter approach was extended with the determination of TBG levels for the lowest 5% of T_4 values. The T_4 /TBG ratio serves as an indirect measure of the free T_4 concentration (which cannot be determined directly in dried blood spots). In contrast to most screening programs, in which TSH levels are determined for the lowest 10% of T_4 readings, TSH levels are measured for the lowest 20% of T_4 values. In this way, the Dutch screening program provides unique information about the prevalence of CH-C.*[1]

Such an approach cannot be performed in countries that have based neonatal screening on blood TSH values. However the merit of the Dutch group had been to adapt the neonatal CH screening in order to be able to detect CH-C. They have shown an unexpectedly rather high frequency of central hypothyroidism at birth. From a clinical point of view the issue is important as many of these children are at risk for neuropsychological disorders and appropriate diagnosis would have been missed or delayed.

There are obvious limitations to the use of the TRH-stimulation test in infants: non-availability of the product in many countries, difficulties in establishing the normal pattern of TSH response, and possible variations in relation to age. Importantly, the subset of patients with MPHD and anatomical defects at the MRI have a serum TSH response distinctly different from the control group. The group of patients with type 2 response showing a flat response due to an impaired release most frequently did not show MRI abnormalities, had a lower incidence of MPHD, and a male predominance. Some had isolated deficiency of unknown origin.

Congenital hypothyroidism can often be diagnosed on a set of clinical symptoms without TRH testing; however it is frequently delayed if midline defects are not present. In patients with an abnormal newborn screen suggestive of CH-C, in whom a TRH test cannot be administered, treatment with thyroid supplementation should be considered throughout the infancy until the diagnosis is established later in life.

In this interesting study the abnormal neonatal screen was the first sign in over 90% of the identified cases with MPHD. It appears that the TRH stimulation test may aid in differentiating CH-C from other diseases in a context of newborn screening with low false-positive rates.[2]

Raphaël Rappaport, MD

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Effect of Levo-thyroxine Treatment on Weight and BMI in Children with Acquired Hypothyroidism

Lomenick and colleagues performed a retrospective analysis of children with the diagnosis of hypothyroidism evaluated in their clinics between July 1995 and July 2006. These authors sought to determine short-term and long-term changes in weight with levo-thyroxine treatment of hypothyroidism. Inclusion criteria were met by 68 subjects, ie under 18 years of age at the time of initial assessment, diagnosis of acquired hypothyroidism, initiation of levo-thyroxine treatment at the first clinic visit, and seen at least once in follow-up. History, physical exams, and laboratory data were obtained from the medical records. Subjects were examined for weight to the nearest 0.1 kg and height to the nearest 0.1 cm as well as BMI. Subjects were divided into 2 groups based on their weight at the second clinic visit compared to their weight at the initial visit; those who lost weight (Group 1; n=21) and those who had no change in weight or who gained weight (Group 2; n=47). Variables were assessed at baseline, first follow-up visit after starting treatment, first visit 2 years after starting treatment, and the first visit 4 years after starting treatment.

The degree of hypothyroidism was variable (TSH 5.5 – 1600 µU/mL) and 81% of the subjects were female. There were no differences in mean age, weight, height, or BMI at baseline between Groups 1 and 2. Children in Group 1 had more severe hypothyroidism with an initial mean TSH of 414 vs 41.4 in Group 2. As anticipated, mean TSH decreased (147 – 5.0 µU/mL) from the initial visit to the first follow-up (an average of 4.4 months after starting treatment). The decrease was not associated with a significant change in mean weight, mean weight percentile, weight z-score, BMI, BMI percentile, or BMI z-score. Mean weight loss in Group 1 children was 2.3 kg which was not significant from baseline.

Thirty subjects had at least 2 years of follow-up. During this interval BMI percentile did not change significantly nor did BMI z-score, weight percentile, or weight z-score. Nineteen children had 4 years of follow and again there was no significant change in BMI percentile, BMI z-score, weight percentile, or weight z-score. Thirty-nine of the 68 subjects were classified as overweight or obese initially (based on BMI). These children exhibited no change in weight or BMI from baseline to the first follow-up. At the second visit (first follow-up) significant correlations were found between initial TSH and change in weight percentile, BMI, BMI z-score, and BMI percentile. After 2 years the initial TSH was negatively correlated with BMI percentile and after 4 years there was a trend toward a correlation between initial TSH and change in BMI percentile.

The authors pointed out that the association between hypothyroidism and weight gain is well described in pediatric textbooks including textbooks on endocrinology and pediatric endocrinology. Indeed practitioners evaluating overweight children often request thyroid tests and prescribe levo-thyroxine for mild hypothyroidism in hope of assisting with weight loss. The current study does not support the notion of hypothyroidism as a cause of obesity and the authors suggested that practitioners should not expect significant changes in weight after treatment in most children with hypothyroidism.

Lomenick JP, El-Sayyid M, Smith WJ. Effect of levo-thyroxine treatment on weight and body mass index in children with acquired hypothyroidism. J Pediatr. 2008:152:96-100.

Editor's Comment: *Lomenick and colleagues have performed a very valuable study. Pediatric endocrinologists who receive referrals from primary care physicians of overweight children with slight elevations in TSH levels are well aware that treatment of such subclinical hypothyroidism rarely achieves significant weight loss. Despite the retrospective nature of this manuscript, it provides significant and important supporting evidence for discouraging unrealistic expectations in families whose overweight children have mild elevations in TSH. This manuscript should be mandatory reading for all physicians who hold out such hope to children or who make referrals to pediatric endocrinologists of such children. The pediatric endocrinology community should congratulate Lomenick and his colleagues and thank them for such a timely manuscript.*

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Geographic Distribution of Childhood Diabetes and Obesity: Workforce of Pediatric Endocrinologists

Lee and associates determined the geographic distribution using the American Board of Pediatrics (ABP) list of pediatric endocrinologists (board certified, less than 65 years of age) by state and data from the National Survey of Children's Health (NSCH). The estimates from the NSCH were obtained by a nationally representative cross-sectional random digit telephone survey of households with children younger than 18 years of age. A single question was asked, "Has a doctor or healthcare professional ever told you your child has diabetes?" The weighted number of children with diabetes was then calculated for geographic divisions in regions of the US (Northeast, Midwest, South, and West). Type 1 and type 2 diabetes prevalence were not separated; the BMI was calculated using CDC growth charts and based on parental reported weight and height, and only obesity (BMI $\geq 95^{th}$ percentile) was utilized in this analysis. Separate ratios of children to pediatric endocrinologists for diabetes and obesity were calculated by dividing the estimated number of children with these disorders by the census region and division. In addition, to determine the extent to which variation and disease prevalence versus pediatric endocrinologist supply affected the differences in geographic ratios, the observed ratios were compared under "index" conditions of greater supply and equitable distribution of pediatric endocrinologists. This calculation assumed that the ratio of child population to endocrinologists for each state would be similar to the state with the largest supply, Massachusetts. Then the ratio of obese children to pediatric endocrinologists was recalculated and the proportion of the observed ratio that would have been attributed to differences in supply was determined.

The authors determined there are an estimated 229,240 children with diabetes and 798 board certified pediatric endocrinologists in the US. The ratio of children with diabetes to board certified endocrinologists is therefore 290:1. Considerable variation by region was seen as the ratios in the Midwest, South, and West were more than double that in the Northeast. There are 17,441 obese children for every board certified pediatric endocrinologist and a 19-fold difference between the highest and lowest ratios per state. Overall the difference between index and observed ratios attributable to supply is 57% for children with diabetes and 69% for children with obesity. In order to reach the index ratios for children with diabetes an additional 2,091 pediatric endocrinologists are needed, and an additional 1,518 pediatric endocrinologists are needed to care for the children with obesity in the US.

The authors noted that although there are benchmarks for the numbers of children in the population per healthcare provider, there are no ideal benchmark ratios for children with chronic diseases to pediatric subspecialists. Given that the average waiting time to see an endocrinologist is approximately 9 weeks, that many board certified pediatric endocrinologists spend only 62% of their time in direct patient care, that annually approximately 76 pediatric endocrinologists have entered the workforce (since 1997), the overall supply will unlikely meet the rising demand due to increasing number of children with diabetes in the US. Suggestions were made for organizing healthcare for diabetes and obesity, including an alternative model of a diabetes team led by a nurse practitioner in consultation with a pediatric endocrinologist may need to substitute for the American Diabetes Association (ADA) recommended diabetes team led by a pediatric endocrinologist. In addition, general pediatricians will need to be taught how to screen, evaluate, and manage obese children while reserving referrals to subspecialists for those for whom specific endocrinological abnormalities are identified.

Lee JM, Davis MM, Menon RK, Freed GL. Geographic distribution of childhood diabetes and obesity relative to the supply of pediatric endocrinologists in the United States. J Pediatr. 2008;152:331-6.

Editor's Comment: *The information presented in this manuscript is not surprising to pediatric endocrinologists who have seen their patient populations grow beyond the level of comfort for providing optimal subspecialty patient care. It is important to note that this particular study was limited to diabetes and obesity and did not include children with other endocrine abnormalities. Thus, the supply of pediatric endocrinologists is much less than that presented for 2 of the most common referrals. It is unfortunate that supply and demand economics are not applied to the care of children with pediatric endocrine disorders. Reimbursement for multidisciplinary diabetes care remains low while that for managing obesity is non-existent in many instances. This manuscript did not address ways in which the supply of pediatric endocrinologists might be augmented, but rather dealt with some suggestions for how a different approach to the care of these children might be entertained. Creative pediatric endocrinologists are called upon to devise creative models for the care of these children which recognize the obvious disparity between index and observed workforce ratios. Such solutions will be mandatory given the rising incidence of diabetes and obesity in our population.*

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